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AN INVESTIGATION OF THE TAXONOMIC STATUS OF ANOPHELES PERPLEXENS LUDLOW, 1907

R. EDWARD BELLAMY, SENIOR SCIENTIST[1,2]

INTRODUCTION

It is the purpose of this paper to present certain data that have come to light on the question of the validity of *Anopheles perplexens* Ludlow, a form too little studied since Howard, Dyar, and Knab (1917) synonymized the species with *Anopheles punctipennis* (Say).

A. perplexens was well named, as perplexity and confusion have plagued our various attempts to understand this mosquito since Ludlow (1907) described the species from a single adult female which she received in a collection from Camp Roosevelt, Mt. Gretna, Pennsylvania. This specimen occasioned Miss Ludlow considerable perplexity, and she stated in the article in which she published the original description, "The general colouring at once suggested one of the *sinensis* group, and it occurred to me that some joke had been perpetrated, so I wrote the collector, asking if it were possible that any Philippine mosquitoes had gotten in with these. He, however, said it was quite impossible, as he had no P. I. mosquitoes with him when this collection was made. No specimens resembling this had previously been received from the U. S., so that I was somewhat reluctant at first to accept it, but as closer study shows it to be an *Anopheles* (as restricted by Theobald), and none of that genus has been received from the P. I., I have decided to publish it."

Dyar and Knab (1908, 1909) refused to accept Ludlow's species as of American origin, and Ludlow (1909a), herself, with weakening conviction, confessed to a belief that ". . . a mistake may have been made in the habitat of this specimen, and that it probably is a Philippine mosquito; . . ." After Mr. Knab had had a chance to examine the type specimen in question, Ludlow (1909b) published a note in which she quoted *his* opinion that the type of *perplexens* was merely an aberrant specimen of *A. punctipennis*, but she included notes which suggest that she did not share this opinion. Miss Ludlow must not have considered that by quoting Mr. Knab's opinion she had synonymized her species with *punctipennis* because in a subsequent publication (Ludlow, 1914) she still treated *perplexens* as a species. Theobald (1910), in treating American anophelines, also recognized *perplexens* as a species.

In listing the synonymy of *A. punctipennis*, Howard, Dyar, and Knab (1917) credit Ludlow (1909b) with synonymizing *perplexens* (as suggested above, a perusal of Ludlow's paper leaves much doubt that she was actually proposing such a synonymy). In any case, Howard, Dyar, and Knab (1917) placed *perplexens* in strict synonymy with *punctipennis*, and to support this measure, pointed out that they had examined dark specimens such as the type of *perplexens* Ludlow "from widely separated localities, so that they do not even represent a local race but are merely an extreme in the ordinary line of variation."

Russell (1925), in his study of the larval characters of *Anopheles* in Lee County, Georgia, found that "four *A. punctipennis* adults were of the variety sometimes called '*A. perplexans*'." King, Bradley, and McNeel (1939) refer to central Florida specimens of *punctipennis* as ". . . possibly the form described as *A. perplexens* (Mt. Gretna, Pa.)." Lawlor (1940) described variations in the ova of *A. punctipennis* from Jenkins County, Georgia, and states, "All of the *punctipennis* females which laid eggs" . . . "were the typical, clearly marked, large-spotted type," but that "smaller, darker specimens found in abundance at Magnolia Springs did not lay eggs in the laboratory."

Vargas (1941) in studying the problem of

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[2]Accepted for publication November 9, 1955.

varieties of *A. punctipennis* concluded that three varieties compose the species: (1) the typical form designated by him as "*typicus*", occurring mainly in eastern North America (but with locality records from Mound, Louisiana, and Mission, British Columbia), (2) a darker form occurring in Mexico and the western United States (and locality records from Wilson Dam, Alabama, and Ottawa, Ontario) which he described as *Anopheles punctipennis stonei*, and (3) the variety *perplexens* Ludlow represented solely by the poorly preserved type female in the U. S. National Museum. While several studies have treated variation in the "*punctipennis* complex" since Howard, Dyar, and Knab synonymized *perplexens* with *punctipennis*, Vargas' is the only one in which *perplexens* is accorded even varietal status; although Russell, *et al.* (1946), apparently following Vargas, include *perplexens* as a variety of *punctipennis* in a key to adults.

Roth (1945) encountered melanistic adults of *A. punctipennis* from Tennessee, North Carolina, and Georgia, and suggests further study to determine "whether any relationship exists between larvae with simple antepalmates and adults with repressed costal markings."

Ross (1947) mentions *punctipennis* adults from Illinois in which the light wing spots are much reduced, others in which these spots are slightly reduced, etc. A large range of these types was taken in a single collection from one locality. He states, "Our collections indicate that there is no particular significance to these variations."

Russell (1925), King, Bradley, and McNeel (1939), and Roth (1945) have all noted a relationship between melanistic (*perplexens* type) adults of the *punctipennis* complex and fourth stage larvae which have hair 2 predominantly single on abdominal segments IV and V. The writer, while employed as Malaria Field Biologist in the Georgia Department of Public Health, 1937–1942, also observed this relationship in collections of *Anopheles* from a number of Georgia counties. Furthermore, it was observed that the "*perplexens* type" larvae (hair 2 on abdominal segments IV and V predominantly single) were to be encountered only in highly calcareous waters, characteristically in the crystal clear, limnocrene limestone springs that are generously distributed through the karst topography areas of south Georgia and north and central Florida.

The material of the *punctipennis* complex examined in the present study is in the main from Baker and Calhoun Counties, Georgia, but does include some material from other Georgia counties and a few specimens from other states. Two forms are recognized in this material. The first of these as an adult is somewhat larger on the average than the second, has a brighter wing, and is generally lighter in color. Its larvae inhabit floodplain pools and turbid hog wallows or roadside puddles. They also occur in ditches and along the edges of slow streams. In spring and fall they may be collected in small numbers in larger ponds. This form is doubtless the most abundant anopheline of the Appalachian Piedmont, and, on the whole, probably the most abundant in eastern North America. There would seem to be little doubt that if Say's (1823) original description referred to any member of what is now recognized as the *punctipennis* complex it was to this form. The egg of this mosquito is quite characteristic.

The second form is smaller and darker as an adult and many specimens show a considerable reduction in the amount of white or yellowish white on the wings, the large light costal spot of *punctipennis* often being quite small and occasionally almost entirely lacking. The larvae occur in clear calcareous springs and in the "spring runs" that issue from these. The egg of this smaller, darker mosquito is distinctly different from that of the larger form discussed above. From Ludlow's original description of *perplexens* and the comments on the type offered by other authors, it is relatively certain that the type of *perplexens* Ludlow is an extremely melanistic individual of the second form mentioned here.

In keeping with the above, I am treating the larger, lighter mosquito as *punctipennis* (Say) and the smaller, darker form as *perplexens* Ludlow; and for reasons provided later these forms shall be treated as species. Some of the differences between *punctipennis* and *perplexens* are indicated in the foregoing introductory and historical paragraphs; these and other differences are illustrated in more detail by the data presented in the following section. While the differences noted are mainly morphological, certain physiological characteristics are also discussed.

MATERIALS, METHODS, AND RESULTS

After discovery (in February 1949) that *perplexens* type females deposit ova that are distinctly different from *punctipennis* ova, it was possible to segregate material of the two species. The procedure was to collect adult females in the wild and isolate them in the laboratory in lantern chimney cages placed over bowls of water. When a batch of ova was deposited by an isolated female, the identity of the ova (and therefore of the parent female) was readily established on microscopic examination of the ova. In some cases the hatchlings from a batch of *punctipennis* ova were kept segregated and were reared as an isolated batch of specimens but in other instances were pooled for rearing with hatchlings from other batches of *punctipennis* ova. By handling the identified batches of *perplexens* ova in the same fashion it was simple to segregate material of the two species at all times.

In this fashion something over 15,000 *perplexens* ova and almost 50,000 *punctipennis* ova were obtained between February 1949 and July 1950.

From these ova a few thousand adults of each species were reared. Some hatched and unhatched ova, representative larvae of all stages, pupal exuviae, and adult males and females were preserved. In the hope of establishing self-perpetuating colonies of the two species most of the reared adults were introduced into separate cages, and in order to obtain as many adults as possible for this purpose most of the larvae and pupae were reared and relatively few preserved.

Morphological characters of the ova, fourth instar larvae, pupal exuviae, and adults of *punctipennis* and *perplexens* were studied and compared. First, second, and third stage larvae were not studied.

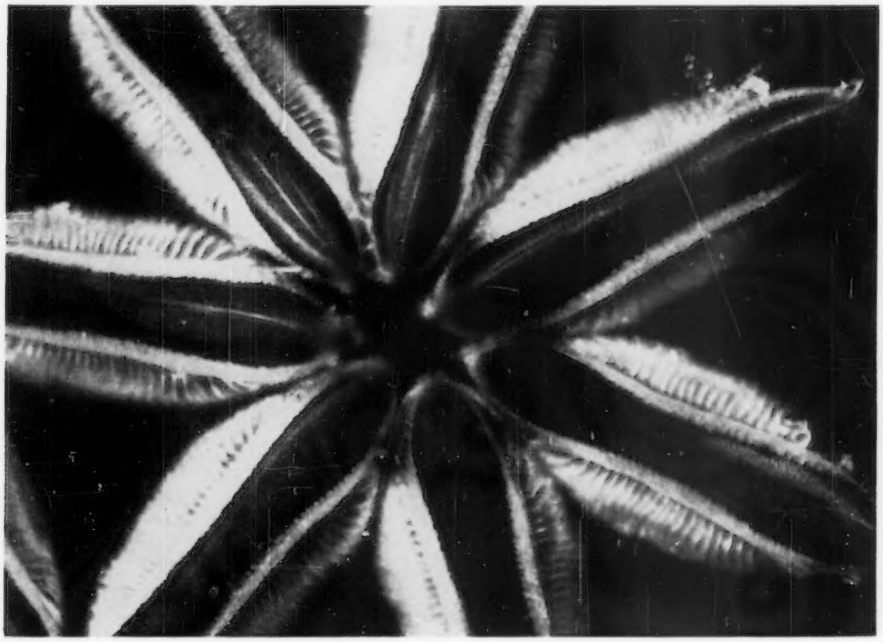

FIG. 1. Photomicrograph of typical ova of *A. punctipennis* (Say). Deposited by specimen 626 collected in Baker County, Ga., 14 February 1950.

Ova

Figure 1 is a photomicrograph of typical ova of *A. punctipennis*, and figure 2 a photomicrograph of typical *A. perplexens* ova. The simplest and most certain distinction between *punctipennis* and *perplexens* is the difference in their ova as illustrated in the photomicrographs. There is characteristically a smooth-edged, elongate oval or slipper-shaped exposure of endochorion dorsally on *punctipennis* ova, the smooth, sweeping edges of the exochorion being continuous with a very high regular (even) frill. In *perplexens* ova there is a much smaller area of exposed endochorion, a narrow and somewhat irregular zone between the floats broadening slightly at either end. As in *punctipennis* ova, the frill is continuous along the edges of the exochorion, but is not high and follows the wavy course of the edge of the exochorion. Comparative measurements of 50 ova of each of the two species are given in table 1.

The ova of *A. punctipennis* were correctly figured and described by Mitchell (1907). The mislabelling of Miss Mitchell's drawings of ova of *A. punctipennis* and *Anopheles crucians* Wiedemann in Howard, Dyar, and Knab (1913, vol. 2, pl. 147) was pointed out by Bellamy and Repass (1950). The description of the egg of *A. punctipennis* provided by Howard, Dyar, and Knab (1917, vol. 4, p. 1013) is obviously based on the mislabelled figure in volume 2. Thus the description these authors offer for the egg of *punctipennis* is based on Miss Mitchell's drawing of a *crucians* egg. As the egg of *perplexens* is superficially similar to that of *crucians*, the description given by Howard, Dyar, and Knab for the egg of *punctipennis* is loosely descriptive of the egg of *perplexens* but not of the egg of *punctipennis*. Herms and Frost (1932) provide figures of *punctipennis* ova deposited by California specimens, and Aitken (1945) also describes and figures ova of California *punctipennis*, including an anomalous egg type constituting part of a batch deposited by one female. Lawlor (1940)

figured usual and "unusual" *punctipennis* ova deposited by Georgia adults. Ova of *A. perplexens* have not been described or figured previously.

Fourth Stage Larvae

By studying slide mounted specimens that had been reared from known ova, the chaetotaxy of fourth instar *punctipennis* larvae was compared to that of fourth instar *perplexens* larvae. About 15 pairs of head hairs, 41 pairs of thoracic hairs, and the 12 to 14 pairs of hairs on each of the first seven abdominal segments, as well as a few hairs on the posterior part of the abdomen, were examined by microscope on two specimens of abdominal segments IV and V of fourth stage larvae of the *punctipennis* complex is a condition associated with *perplexens* type adults. Hair 2 is characteristically bifurcate on the corresponding segments of fourth stage larvae of *punctipennis* (Root, 1924; Russell, 1925; Bradley, 1936; King, Bradley, and McNeel, 1939; King and Bradley, 1941; Ross and Roberts, 1943; Carpenter *et al.*, 1946; Ross, 1947). The condition of hair 2 on abdominal segments IV and V was accordingly selected for comparing fourth instar *perplexens* and *punctipennis*. The batches of larvae rearing from known ova of the two species provided excellent material for a statistical check of this character.

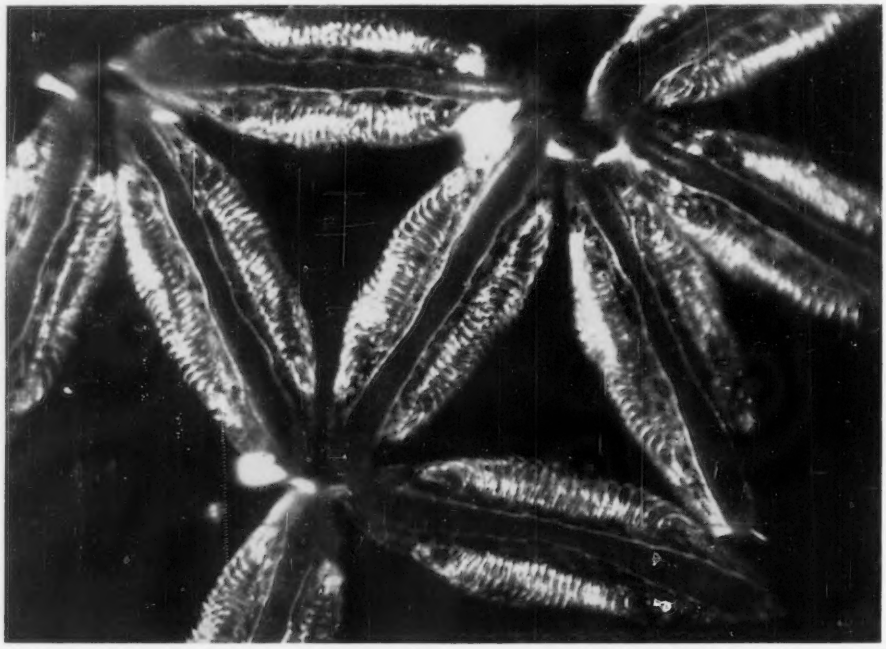

FIG. 2. Photomicrograph of typical ova of *A. per-plexens* Ludlow. Deposited by specimen 401 collected in Calhoun County, Ga., 13 June 1949.

each species. The number of branches and the mode of branching of each hair was recorded. Analysis showed that almost without exception there was overlap in the ranges in the number of branches of the corresponding hairs of the two species and in most cases these ranges overlapped broadly. Since this overlap in the branching of the various hairs was so general (even though recorded for right and left sides of only two specimens of each species), it appeared that a statistical treatment would be necessary to demonstrate a difference in the chaetotaxy of the fourth stage larvae of the two forms.

As stated earlier, several workers have noted that unbranched antepalmate hairs (hair 2) on

A total of 1,620 fourth stage larvae of each species was examined under a binocular dissecting microscope (magnification: about 54x) and the condition of hair 2 on abdominal segments IV and V was recorded for each specimen. Since hair 2 occurs at four positions on segments IV and V (IV right, IV left, V right, and V left), the condition of 6,480 *punctipennis* hairs and 6,480 *perplexens* hairs was recorded. Representative descriptions from my notes on the condition of this hair are as follows: "single"; "frayed at tip"; "split near tip"; "split $\frac{1}{4}$ from tip"; "split $\frac{1}{3}$ from tip"; "split at midlength"; "split $\frac{1}{3}$ from base"; "split $\frac{1}{4}$ from base"; "split near base"; "weaker 3d (middle) branch near base"; "triple

¼ from base"; "4-branched ¼ to ⅓ from base"; "split near base and weak 3d branch near tip of anterior bifurcation"; etc. Obviously, considerable variety was encountered in the nature and degree of branching. Therefore, in the analysis of these descriptions, those branches described as arising at midlength or more proximad on any hair were included, but branches described as originating beyond midlength were disregarded. The results are presented graphically in figure 3. When this character alone is considered, there is only a small amount of overlap between *perplexens* and *punctipennis* as figure 3 shows.

generalization, the branching of this hair on the specimens studied showed much promise as a differentiating character, in *perplexens* being 7 to 17 branched (average: 9.6 branches) and in *punctipennis* 5 to 8 branched (average: 6.2 branches). All counts were made on slide mounted larvae examined under a magnification of about 440 diameters. Prothoracic hair 14 is too small to be studied by dissecting microscope on living larvae, and on some mounted larvae may be difficult to locate. Since this hair is not dorsal and is more anterior in position than ventral, it is more satisfactorily studied on shed

TABLE I

NUMBER OF FLOAT RIDGES AND DIMENSIONS OF REPRESENTATIVE OVA OF *Anopheles perplexens* AND *Anopheles punctipennis*

EGG FEATURES[b]	OVA OF *A. punctipennis*						OVA OF *A. perplexens*					
	Batch[a] 653A	Batch 663A	Batch 720A	Batch 754A	Batch 773A	AVERAGE OF FIVE BATCHES	Batch 613A	Batch 632A	Batch 645A	Batch 647A	Batch 650A	AVERAGE OF FIVE BATCHES
a. Length	611	588	578	524	537	568	669	620	620	598	653	632
b. Greatest breadth including floats	215	213	210	207	221	213	221	225	224	224	232	225
c. Float length												
Right	368	399	388	337	373	373	439	406	397	399	475	423
Left	368	395	393	338	366	372	440	402	391	400	477	422
d. Number of float ridges												
Right	24.8	28.3	26.7	21.8	27.1	25.7	25.6	27.4	25.7	25.0	28.1	26.4
Left	23.9	27.8	25.9	21.5	27.3	25.3	25.9	26.3	26.1	24.6	27.7	26.1
e. Width of exposed endochorion at broadest point anterior end	90	68	87	77	72	79	75	58	66	65	65	66
f. Width of exposed endochorion at broadest point behind floats	70	59	72	63	59	65	63	43	59	51	58	55
g. Width of exposed endochorion at narrowest point behind floats	65	54	62	57	55	59	41	22	33	26	32	31
h. Distance between right and left floats	106	83	99	91	94	95	97	87	88	90	92	91
Ratio of width of exposed endochorion at narrowest point between floats (g) to egg length (a)	.106	.092	.107	.109	.102	.104	.061	.035	.053	.043	.049	.049

[a]Each batch consists of ten ova chosen at random from the first lot deposited in captivity by a particular female (Each batch was deposited by a different female.)

[b]Measurements, except (d), were made by ocular micrometer and are given in microns (one scale div. = 13.8 microns.) Figures are means in every case.

It is not improbable that, just as with hair 2 on abdominal segments IV and V, a statistical analysis of the number of branches of various other hairs would demonstrate marked average differences between larvae of the two species. For instance, the number of branches of prothoracic hair 14 may serve as a character to help differentiate *perplexens* from *punctipennis* larvae. This hair was examined on right and left sides of 20 fourth stage larvae of each species, and while the series examined is too small to support

fourth stage larva skins than on whole mounts of the larvae.

In the hope of finding a character which would permit absolute separation of the larvae, head capsules of several representative fourth instars of both species were dissected and studied. A preliminary comparison of various chitinized head structures including the antennae, mandibles, palps, mentum, submentum, and prementum revealed no reliable character that could be used to differentiate the two species.

Pupae

The pupal chaetotaxy of *A. punctipennis* was compared to that of *A. perplexens* by examining pupal exuviae obtained in lots of material reared from identified ova. The cast pupal pelts were dissected and mounted in euparal on microscope slides. The dissections were made according to the method recommended and figured by Knight and Chamberlain (1948) except that the cephalic shield was always freed from the thoracic portion of the cephalothoracic mass in order that the cephalic setae could be seen more clearly. The 12 pairs of cephalothoracic setae and 88 pairs of abdominal setae were examined by compound microscope on the slide mounted pupal exuviae of two specimens of *perplexens* and two of *punctipennis* (all four were females). The number of branches and the nature of the branching was recorded for each hair on each specimen. As was the case with fourth stage larvae, the overlapping in the branching of the corresponding hairs was so general that it appeared unlikely that differences could be shown other than statistically. The material examined was insufficient to permit a statistical analysis.

Ten pupal pelts of *perplexens* (five males and five females) were checked against the pupal characters given for *punctipennis* by Darsie (1949) and against those given by Penn (1949). As certain points of divergence were noted, the pupal exuviae of ten specimens of *punctipennis* (five of each sex) were checked against the keys provided by Darsie and by Penn, and the lack of agreement was found to be nearly as great for *punctipennis* as for *perplexens*. On the characters given in Darsie's key, nearly all of the specimens of both species would key to *punctipennis*, but at couplet 6 of his key nearly all corresponded properly to some of the characters given and failed to correspond to others. Therefore, if these specimens had been of unknown derivation, considerable doubt would have existed (at least for several of them) as to whether they should have been keyed to *punctipennis* or to *quadrimaculatus*. The pattern was not the same for all specimens, but lack of agreement was most evident for the first and fourth characters of Darsie's key couplet 6, and some failure to correspond properly with the first character of

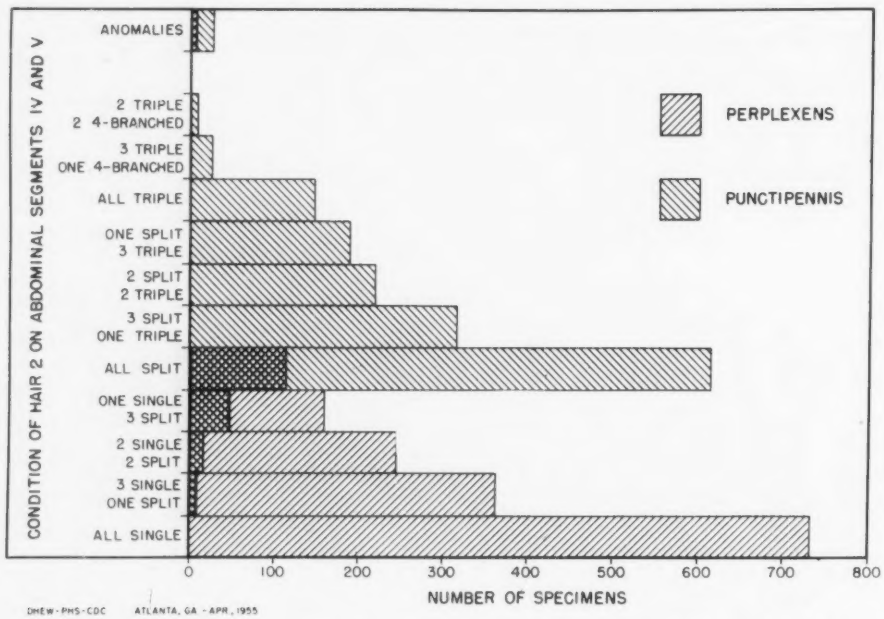

FIG. 3. Graphic analysis of branching of hair 2 on abdominal segments IV and V of 1,620 fourth instar larvae of *A. perplexens* and 1,620 fourth instar *A. punctipennis*.[3]

[3]All specimens were reared from identified ova and were kept segregated before hatching and during rearing. Nearly all larvae were alive when examined. The progeny of at least a dozen females of each species are included. "All single" signifies hair 2 unbranched both at right and at left position on segment IV and at right and left positions on segment V; "3 single, one split" signifies a bifid hair 2 at any one of these positions and at the other 3 positions an unbranched hair 2, etc. (For purposes of this analysis, all branches originating on hair 2 at midlength or more proximad are counted, while branches arising beyond midlength are disregarded.)

his couplet 3 and the third of couplet 5 was noted.[4]

Several of the specimens of both species fail to key to *punctipennis* in Penn's key which is not reinforced with the multiplicity of characters presented by Darsie (my material is all of Georgia derivation while the material examined by Penn was from New York, California, and Mexico). The majority of the Georgia *perplexens* and *punctipennis* examined lie on intermediate ground at couplet 3 of Penn's key and one specimen of *perplexens* clearly departs from *punctipennis* in the key at this point. About a fourth of the *punctipennis* fail to correspond properly at couplet 6. Again, at couplet 9, nearly all of the specimens of both species are intermediate while some, particularly *perplexens*, definitely key away from *punctipennis*. Furthermore, all the Georgia material fails to correspond with certain characters in the first part of couplet 10; however, the key is very confusing at this point since the first and third statements given by Penn as applicable to *punctipennis* are inconsistent with his figure of this species, and the second statement contradicts information he gives in his more detailed description of *punctipennis*.

Adults

Size and Coloration.—As mentioned above, several authors have encountered small dark specimens of the *punctipennis* complex in which the light costal wing spot at the apex of the subcosta (the "subcostal spot" of Christophers [1913] and Root [1926]) was generally of small size, and some of these authors recognized such specimens as being of the "*perplexens* type." In order to test the hypotheses: (1) that *punctipennis* is the larger mosquito, and (2) that the subcostal spot is smaller in the wing of *perplexens*, measurements of representative material of the two species were made.

Wing length was selected as a criterion of total size, since the length of a wing is not likely to be affected by post-mortem changes. A wing was clipped from each specimen to be measured (unless damaged, the left wing was used) and was mounted in euparal, via essence of euparal, on a microscope slide. Using an ocular micrometer in a 10X ocular in combination with a 48 mm. objective, the distance from humeral cross-vein to wing tip was measured on each specimen and recorded as wing length. For those specimens whose wing length exceeded the total scale of the ocular micrometer, summation of two measurements (*i. e.* (1) humeral cross-vein to point of divergence of veins R_2 and R_3 plus (2) point of divergence of R_2 and R_3 to wing tip) was necessary. Specimens selected for measurement include a few preserved wild adult females whose identity was established by the ova they deposited; other specimens were arbitrarily taken as encountered in the various lots of preserved reared material of the two species (*i. e.* in choosing these representative specimens no selection for total size or size of wing spot was exercised). As explained later, most *punctipennis* larvae were reared in untreated tap (deep well) water, while the *perplexens* larvae were reared in water from a nearby calcareous stream. Therefore, only part of the reared material of the two species is comparable.

The average wing length of 17 wild females which had deposited *perplexens* ova was 4.109 mm (range 3.640–4.594 mm), while the average length of wing of 23 wild specimens which deposited *punctipennis* ova was 4.129 mm (range 3.455–4.770 mm). The difference in wing length was greater for 25 females of each species reared in creek water from identified ova (for each species, five progeny of each of five females): average *perplexens* wing length 3.575 mm (range 3.286–3.922 mm) and average *punctipennis* wing length 4.123 mm (range 3.677–4.770 mm). A similar difference was observed in the average wing lengths of 25 males of each species reared in creek water from identified ova (for each species, five progeny from each of five females): average male *perplexens* wing length 3.073 mm (range 2.756–3.357 mm) and average male *punctipennis* wing length 3.741 mm (range 3.138–4.346 mm). The average length of wing of male and female *punctipennis* adults reared in untreated tap (deep well) water from identified ova was considerably less than that of the same species reared in creek water. Again, 25 specimens of each sex representing in each case five progeny of each of five females were measured, and the average female wing was only 3.437 mm (range 3.039–3.922 mm), while the average male wing was 3.463 (range 3.039–3.958 mm). Attempted rearings of *perplexens* in tap water were not successful.

The identity of all the above specimens was confirmed either through the ova they deposited or through rearing from segregated identified batches of ova. Additional specimens of the *punctipennis* complex whose identity was *not* confirmed were also measured. These were wild adult males and some wild females which deposited no ova, and were neither (a) from *perplexens* localities (wild females from these localities usually deposit *perplexens* ova), or (b) from *punctipennis* localities. The average wing length of 5 *punctipennis* complex females from three *perplexens* localities was 3.809 mm (range 3.392–4.311 mm), while the average wing length of 15 female specimens from three *punctipennis* localities was 4.492 mm (range 3.640–5.018 mm). The

[4]The general overlapping of Darsie's key characters (while undoubtedly presenting an accurate picture of his findings) serves to emphasize the difficulty to be encountered in keying pupae of this group of anophelines. Darsie must have been well aware of this as he stresses the extreme similarity of *quadrimaculatus* and *punctipennis* pupae.

average wing length of 29 *punctipennis* complex males from four *perplexens* localities was 3.605 mm (range 3.039–4.028 mm), and the average wing length of 13 males from two *punctipennis* localities was 3.943 mm (range 3.286–4.346 mm).

On each of the wings measured to determine wing length, the subcostal spot was also measured and in the same manner except that the 16 mm objective was used. The extent of this yellowish white subcostal spot along the costa was chosen as the standard measurement. Wing size was quite variable in both species as indicated above; therefore, for comparing the size of the subcostal spot in the wings of the two species, the ratio of the length of the subcostal spot to wing length was calculated. This may also be stated as the percentage of the wing length occupied by the subcostal spot. The average ratios are compared in figure 4.

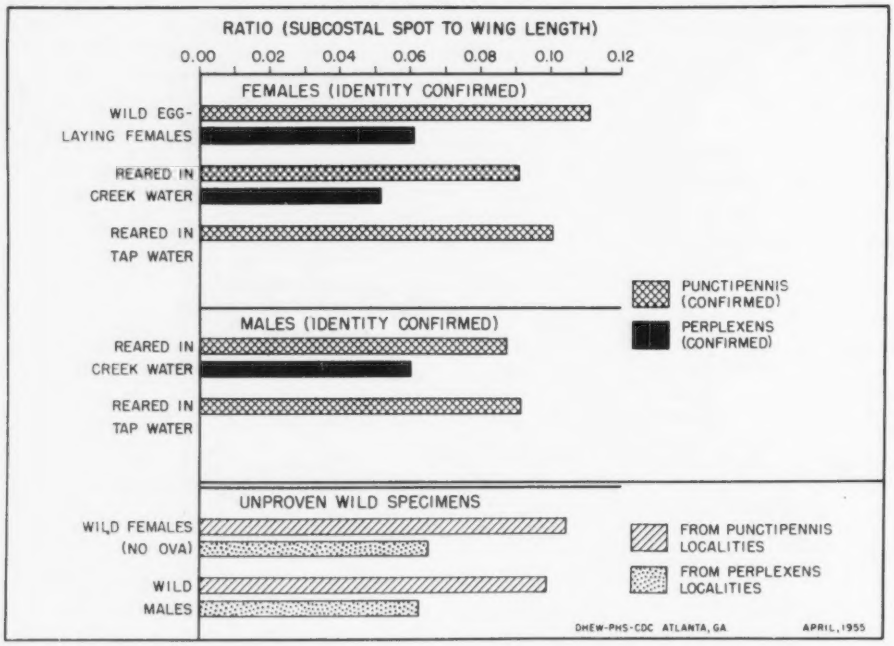

FIG. 4. Ratio of subcostal spot to wing length in adults of *punctipennis* complex.

Morphology of the Mouth Parts, Tarsi, and Genitalia.—Various morphological characters were compared on a few adult male and female representatives of the two species. In all cases the specimens examined were reared from identified ova. The appropriate parts were dissected, macerated in 5 to 10 percent KOH, and mounted in euparal (via acetic acid and oil of cloves).

Females.—The dissected mouth parts, cibarial armature, hypopygium, and spermatheca of representative females of the two species were compared, but without discovery of differences of diagnostic value in any of these structures. The number of maxillary teeth ranged from 14 to 17 in *perplexens* and from 13 to 16 in *punctipennis* (about ten specimens of each species were examined). The type of slide mounts did not permit examination with an oil immersion objective in all cases; therefore, the count of maxillary denticles was not entirely satisfactory.

Males.—The hypopygial characters of *punctipennis* and *perplexens* were compared. Even though the series studied was small (about a dozen specimens of each species), much variation was found in the hypopygia of both *perplexens* and *punctipennis*. The variations observed were of the order of those reported for *punctipennis* by Roth (1944); in fact, the variation was even greater than that found by Roth. While the posteriorly directed arms of the ninth tergite tended to be proportionately longer (as compared to the length of the dorsal pigmented band connecting them) in *punctipennis* than in *perplexens*, neither species was constant and the shape as well as the proportionate length of these arms varied in both. Much variation was found in the number and shape of spines from the dorsal lobe of the claspettes of both *punctipennis* and *perplexens*, and several specimens were asymmetrical. Various combinations of one or two (closely applied or separated) pointed and

apically rounded spines were observed to arise from the dorsal lobe of the claspettes of both species (one *perplexens* male had three apically rounded spines from the dorsal lobe of one claspette and two from the other). It is interesting that the claspette spine arrangements given by Vargas (1941) as typical of his *punctipennis stonei* and of "*punctipennis typicus*" were both represented in the small series of *perplexens* examined as well as among the specimens of *punctipennis*. The number of leaflets on the phallosome was also variable, particularly in *punctipennis*, there being one more leaflet on one side than on the other in several instances (in *punctipennis* the total number of leaflets varied from five to nine, eight being most common; in *perplexens* the number varied from four to eight and six was most common).

Dissected mouth parts and the apical claws and sex comb of the prothoracic tarsus of males of the two species were also compared but differences were not detected.

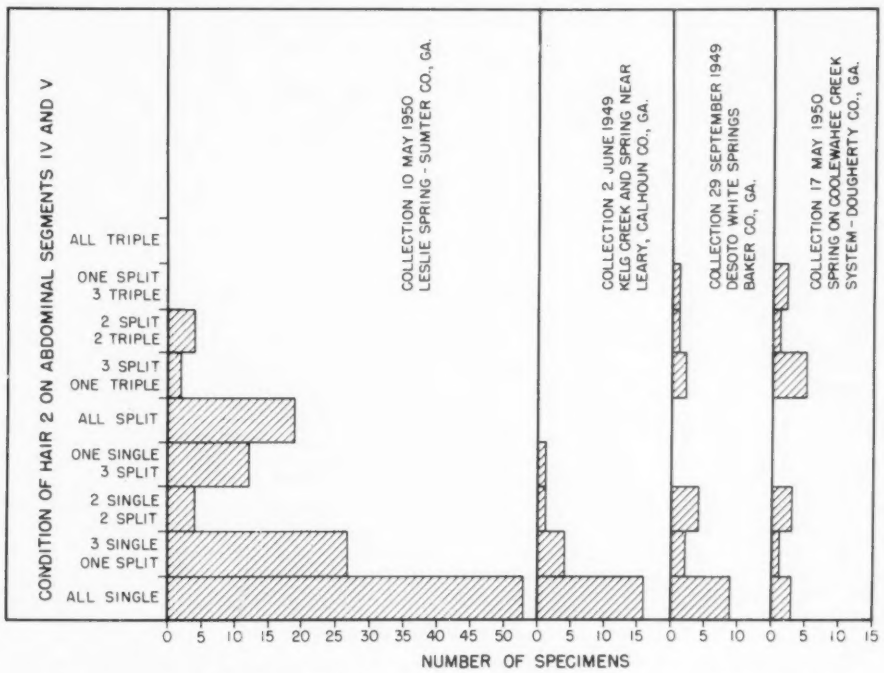
CONDITION OF HAIR 2 ON ABDOMINAL SEGMENTS IV AND V
ALL TRIPLE
ONE SPLIT 3 TRIPLE
2 SPLIT 2 TRIPLE
3 SPLIT ONE TRIPLE
ALL SPLIT
ONE SINGLE 3 SPLIT
2 SINGLE 2 SPLIT
3 SINGLE ONE SPLIT
ALL SINGLE
COLLECTION 10 MAY 1950 LESLIE SPRING - SUMTER CO., GA.
COLLECTION 2 JUNE 1949 KELG CREEK AND SPRING NEAR LEARY, CALHOUN CO., GA.
COLLECTION 29 SEPTEMBER 1949 DESOTO WHITE SPRINGS BAKER CO., GA.
COLLECTION 17 MAY 1950 SPRING ON COOLEWAHEE CREEK SYSTEM - DOUGHERTY CO., GA.
0 5 10 15 20 25 30 35 40 45 50
0 5 10 15
0 5 10
0 5 10 15
NUMBER OF SPECIMENS

FIG. 5. Graphic analysis of branching of hair 2 on abdominal segments IV and V of populations of fourth instar larvae of the *punctipennis* complex from *perplexens* habitats.

PHYSIOLOGICAL CHARACTERISTICS

Ecological Distribution.—As previously stated, *perplexens* is encountered with great regularity in the immediate environs of clear, pool-forming springs that issue through subterranean limestone strata (north Florida and south Georgia). Adults of the *punctipennis* complex found near such springs are mainly small dark specimens with a reduced subcostal spot, and it is thus almost certain that most of these are *perplexens*. The fact that a number of adult females of this appearance collected about such locations have deposited *perplexens* ova when brought to the laboratory, greatly strengthens this supposition. Occasional adults from such locations have had the characteristic appearance of the larger, lighter *punctipennis*, and in a few instances the ova obtained from such specimens confirmed that they were *punctipennis*. Still other specimens from these localities have appeared intermediate, but ova obtained from these have generally revealed the ovipositing adults to be *perplexens*. In agreement with this, the *punctipennis* complex larvae, taken from these calcareous springs and the adjacent spring runs, are mostly *perplexens* as the predominantly single condition of hair 2 on abdominal segments IV and V indicates (see figure 5). Figure 5 is a graphic analysis of the condition of hair 2 on abdominal segments IV and V of four aggregations of *punctipennis* complex fourth instar larvae, taken at as many different calcareous springs. The analyses of populations of larvae from *perplexens* localities given in figure 5 should be compared to figure 3.

A type of circular "lime sink hole" with abrupt

almost perpendicular sides sloping down ten to thirty feet to a pool of usually shaded clear water (often with a luxuriant cover of duckweeds) is a feature of not infrequent occurrence in Lee and Crisp Counties, Ga., and Leon County, Fla. These "sink holes" have been observed to harbor larvae which, like the specimens from limestone springs, have the characteristics of *perplexens*. Lime sinks of this type occur in general in the vicinity of the clear calcareous springs which constitute the principal habitat of *perplexens;* the springs and many of the steep-sided lime sinks are obviously directly connected to subterranean water-charged caverns.

ova was very high. In attempting to rear several lots of larvae hatched from *perplexens* eggs, less than a dozen specimens attained the fourth larval instar and only two or three adults were obtained. In these rearings the larvae were kept in pans of tap water and were fed finely ground dog chow daily, the same rearing procedure which at the time was resulting in satisfactory development of *quadrimaculatus* and *punctipennis* larvae without excessive mortality. The source of the tap water used is a deep well. Further attempts to rear *perplexens* larvae were quite successful when the hatchlings were transferred to pans of water taken from a clear limestone spring which

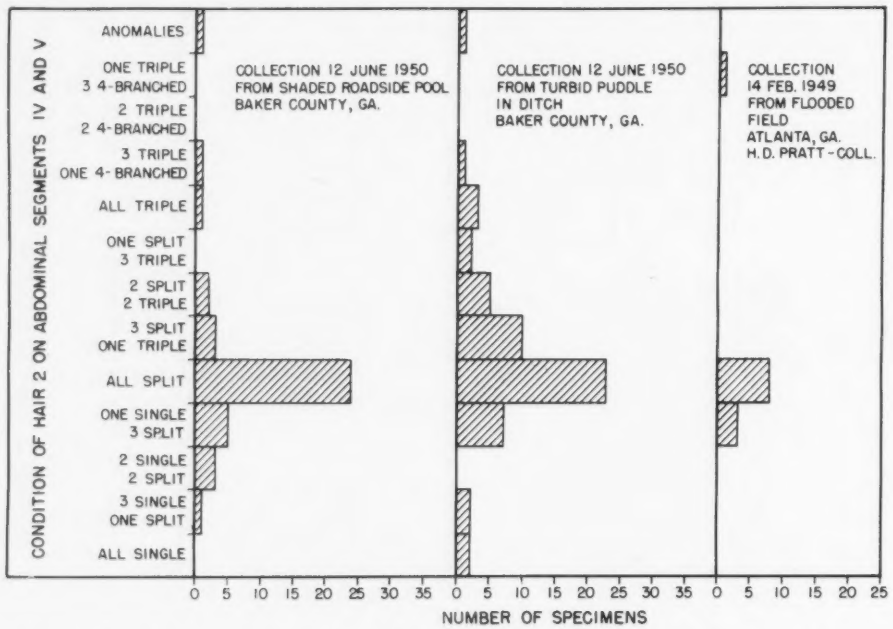
CONDITION OF HAIR 2 ON ABDOMINAL SEGMENTS IV AND V
ANOMALIES
ONE TRIPLE 3 4-BRANCHED
2 TRIPLE 2 4-BRANCHED
3 TRIPLE ONE 4-BRANCHED
ALL TRIPLE
ONE SPLIT 3 TRIPLE
2 SPLIT 2 TRIPLE
3 SPLIT ONE TRIPLE
ALL SPLIT
ONE SINGLE 3 SPLIT
2 SINGLE 2 SPLIT
3 SINGLE ONE SPLIT
ALL SINGLE
COLLECTION 12 JUNE 1950 FROM SHADED ROADSIDE POOL BAKER COUNTY, GA.
COLLECTION 12 JUNE 1950 FROM TURBID PUDDLE IN DITCH BAKER COUNTY, GA.
COLLECTION 14 FEB. 1949 FROM FLOODED FIELD ATLANTA, GA. H.D. PRATT-COLL.
0 5 10 15 20 25 30 35
0 5 10 15 20 25 30 35
0 5 10 15 20 25
NUMBER OF SPECIMENS

FIG. 6. Graphic analysis of branching of hair 2 on abdominal segments IV and V of populations of fourth instar larvae of the *punctipennis* complex from *punctipennis, s.s.*, habitats.

Aggregations of *punctipennis* complex larvae collected from roadside puddles, turbid hog wallows, and floodplain pools appear to be predominantly if not exclusively *punctipennis* as hair 2 on abdominal segments IV and V is usually two or more branched on such specimens. In figure 6, graphs are given of analyses of the condition of hair 2 on these segments for populations of larvae collected at three locations of the kind described above. Figure 6 should be compared to figures 3 and 5.

Behavior in the Laboratory.—Batches of *perplexens* ova deposited by caged wild specimens hatched satisfactorily in the bowls of water in which they were kept in the laboratory, but the mortality of the larvae that hatched from these

was known to be a natural habitat of this form. It was also discovered that *perplexens* would rear excellently in water from the nearby Ichaway-notchaway Creek which is a highly calcareous stream that has entrenched itself in limestone strata. In this connection it is interesting that *punctipennis* also appears to rear more satisfactorily in the creek water; mortality is apparently lower, and, on the average, larger adults are obtained.

Attempts to Establish Cage Colonies.—Numbers of *punctipennis* adults reared from ova were introduced into a (2′ x 2′ x 1½′) cage constructed of wood and 18 mesh hardware cloth. The cage was kept in an insectary room where the temperature was controlled at 78±2° F. and humidity at

85 ± 10% RH. The female adults were permitted to take blood from a rabbit placed in the cage for a few hours several days each week. Cellucotton pads saturated with a 2% solution of corn syrup were available to the mosquitoes at all times. In a few instances small aggregations of ova were obtained in a pan of water placed overnight in the cage, but only a few of these hatched; the others were apparently infertile. Other adults were placed in larger cages kept where they were more exposed to temperature changes, but the mosquitoes were protected by wet towelling draped over one or more of the screen sides of these cages. Again the ova obtained were few although more than a thousand adults were often present in a cage.

Almost identical procedures were used in attempts to establish a cage colony of *perplexens* in cages 2′ x 2′ x 1½′. Infertile ova were obtained several times, both from caged adults kept under the constant conditions of the insectary and from caged specimens kept where they were relatively exposed to diurnal temperature changes. The adults caged where exposed to temperature changes, however, deposited several small aggregations of fertile ova. The cage in which these reared adults were housed was kept blanketed with wet towels to increase humidity. In addition to the cellucotton pad saturated with a dilute solution of corn syrup which was standard for all cages, slices of apple were provided.

Fertile and infertile ova alike that were deposited by the reared *punctipennis* adults were typical for this species. Similarly, the fertile and infertile ova deposited by the reared *perplexens* adults were all characteristic *perplexens* ova. This is consistent with the fact that infertile ova deposited by reared adults of other species (e.g., *quadrimaculatus* and *crucians*) appear identical in each case to the fertile ova deposited by isolated wild adult females of the corresponding species.

DISCUSSION

It is most unfortunate that there is not extant the type material on which Thomas Say based his original description of *punctipennis*, especially since his original description is too vague to dispel doubts as to what mosquito he had before him. Indeed, Say's original description of *Culex punctipennis* suggests some other species (e.g., *A. quadrimaculatus* of authors) than that now commonly recognized by authors as *A. punctipennis*. In spite of this, the general acceptance of Say's name for this common mosquito makes it most desirable that the nomenclatural *status quo* be preserved. Recognition of neotypes by the International Commission on Zoological Nomenclature (and perhaps other official sanction by this body) would permit the selection of a good specimen (preferably an adult female reared from a known egg, with all cast skins including the eggshell preserved) from the approximate type locality of Say's *punctipennis* to stabilize the nomenclature.

The holotype of *perplexens*, though said to be in poor condition (*vide* Vargas, 1941), is at least in existence, reposited at the U. S. National Museum. While it has not been possible for me to see this specimen, Ludlow's (1907) original description and the comments on the type offered by Howard, Dyar, and Knab (1917) strongly indicate that Ludlow's type is conspecific with the form I am treating as *perplexens* in this paper. The need for characters which would permit positive identification of adult females of the latter form (especially as separable from adult females of *punctipennis*) has a special significance in that the type of *perplexens* is an adult female. The average differences (wing length, and particularly, relative size of subcostal spot) shown for wild and reared females of the two forms unfortunately do not serve as a means of positive identification of an individual specimen, and a search for one or more characters which would serve in this capacity was not rewarded with success. Accordingly, the identity of the type of *perplexens* with the form treated here as *perplexens* could not be positively established on the basis of characters now available. However, a careful examination of the wings of this type specimen would undoubtedly show it to fit better in the series of females of the form treated as *perplexens* in this paper than in the series treated as *punctipennis*. Even a dissection of the type would be justified if, in the absence of reliable external characters, good internal characters could be found for distinguishing the adult females of the two forms, but there is at present no indication that internal characters show as much promise as external ones in this respect. As the name, *perplexens*, is available and the form treated as *perplexens* in this paper includes many specimens which correspond to the original description of this species, it is preferable to utilize this name rather than to resurrect another whose accompanying description would not correspond as well; and the proposal of a new name, with the name, *perplexens*, available, would only complicate the nomenclature further.

It is clear that in a fairly large area of south Georgia and north Florida *punctipennis* and *perplexens* inhabit different types of breeding areas with relatively little overlap in their ecological segregation. However, the two forms do occur side by side to some extent, and generally within a mile of a characteristic breeding area of *perplexens* one or more typical breeding areas of *punctipennis* can be found. An examination of the fauna of clear limestone springs throughout the eastern United States will probably reveal that *perplexens* is widely distributed and that accordingly the range occupied in common with *punctipennis* is a great one. In any case *punctipennis*

and *perplexens* do occupy in common a considerable area and appear, therefore, though morphologically similar, to be a pair of sympatric species in the terminology of Mayr (1942: p. 148). Huxley (1942) and Bates (1949) refer to such forms as "cryptic species." It is difficult to think of *perplexens* as an ecological variant when the second generation ova of the two forms are in each case true to type. Furthermore, mixed batches of ova were not encountered; nor have ova intermediate in character been observed among typical *punctipennis* ova deposited by over 150 isolated females or among typical *perplexens* ova deposited by 63 females. One batch of ova deposited by a *perplexens* female was sufficiently atypical to provoke some doubt as to its identity, but subsequent batches deposited by the same female were typical *perplexens* ova.

It is of course conceivable that *perplexens* and *punctipennis* might behave as separate species in south Georgia but intergrade in some other geographic area. Such relationships within a single species are known to exist among the vertebrates (Dice, 1941; Stegmann, 1934) and have been reported for several groups of insects (gall wasps: Kinsey, 1936, 1937; butterflies: Forbes, 1928; etc.). In the absence of data to demonstrate such a relationship, however, it is more conservative to treat the two forms as separate species.

In keeping with the above, two species are recognized in the *punctipennis* complex. The species and literature references pertaining to each are listed below:

1. *Anopheles (Anopheles) perplexens* Ludlow
   Anopheles perplexens Ludlow, 1907 Can. Ent., 39: 267.
   Anopheles perplexens Ludlow. Theobald, 1910, Mon. Culic., Brit. Mus. Nat. Hist., London, vol. 5, p. 7.
   Anopheles perplexens Ludlow. Ludlow, 1914, Disease Bearing Mosquitoes of North and Central Amer., W. I., and P. I. War Dept., Off. Surg. Gen'l., Bull. No. 4, Washington, D. C., 1914, p. 23.
   Anopheles punctipennis (Say) [*partim*]. Howard, Dyar, and Knab, 1917, Mosq. of N. & Cent. Amer. & W. I., Carnegie Inst. Wash., Publ. 159, vol. 4, p. 1009.
   Anopheles punctipennis (Say) [*partim*]. Matheson, 1929, Handbook Mosquitoes N. Am., Springfield, Ill., p. 85.
   Anopheles (Anopheles) punctipennis (Say) [*partim*]. Edwards, 1932, Diptera. Family Culicidae, Bruxelles (In P. Wytsman, Genera Insectorum, Fasc. 194), p. 39.
   Anopheles punctipennis (Say) [*partim*]. King, Bradley, and McNeel, 1939, Mosq. Sou.-East. States, U. S. Dept. Agri., Misc. Publ. 336, Wash., D. C., p. 34.
   Anopheles punctipennis var. *perplexens* Ludlow. Vargas, 1941, Rev. de la Soc. Mex. de Hist. Nat., vol. 2, nos. 2-3, p. 178.
   Anopheles punctipennis (Say) [*partim*]. Roth, 1945, Proc. Ent. Soc. Wash., vol. 47, no. 9, p. 261.
2. *Anopheles (Anopheles) punctipennis* (Say)
   Culex punctipennis Say, 1823, Journ. Acad. Nat. Sci. Phila., vol. 3, no. 1, p. 9.
   Anopheles punctipennis (Say). Say, 1824, Keating's Narr. Exped. St. Peter's River, vol. 2, p. 357. [*fide* Howard, Dyar, and Knab, 1917].
   Culex hyemalis Fitch, 1847, Amer. J. Agri. and Sci., vol. 5, p. 281. [*fide* Howard, Dyar, and Knab, 1917].
   Anopheles punctipennis (Say). Theobald, 1907, Mon. Culic., Brit. Mus. Nat. Hist., London, vol. 4, p. 27.
   Anopheles punctipennis (Say). Howard, Dyar, and Knab, 1912 [1913], Mosq. N. & Cent. Am. & W. I., Carnegie Inst. Wash., Publ. 159, vol. 2, pl. 40, fig. 268 [?] pl. 41, fig. 7, pl. 84; pl. 130, fig. 451; pl. 135, fig. 515; pl. 138, fig. 542; pl. 144, fig. 664; pl. 147, fig. 696 [as *A. crucians* Wied.].
   Anopheles punctipennis (Say) [*partim*]. Howard, Dyar, and Knab, 1917, Mosq. N. & Cent. Am. & & W. I., Carnegie Inst. Wash., Publ. 159, vol. 4, p. 1009.
   Anopheles punctipennis (Say) [*partim*]. Matheson, 1929, Handbook Mosquitoes N. Am., Springfield, Ill., p. 85, pl. 1, fig. 3; pl. 8, fig. 4; pl. 9, fig. 4; fig. 9A, p. 20; fig. 13g, p. 30 [as *A. crucians*].
   Anopheles (Anopheles) punctipennis (Say) [*partim*]. Edwards, 1932, Diptera. Family Culicidae, Bruxelles (In P. Wytsman, Genera Insectorum, Fasc. 194), p. 39.
   Anopheles punctipennis (Say) [*partim*]. King, Bradley, and McNeel, 1939, Mosq. Sou.-East. States, U. S. Dept. Agri., Misc. Publ. 336, Wash., D. C., p. 34, pl. 5c [?].
   [?] *Anopheles punctipennis* var. *typicus* Vargas [*Anopheles punctipennis punctipennis* (Say). Vargas], 1941, Rev. Soc. Mex. Hist. Nat., 2 (2-3) 178, figs. 2, 4.
   [?] *Anopheles punctipennis stonei* Vargas, 1941, Rev. Soc. Mex. Hist .Nat., 2 (2-3): 179, figs. 1, 3.
   Anopheles punctipennis (Say) [*partim*]. Roth, 1945, Proc. Ent. Soc. Washington, 47(9): 261, pl. 30, figs. 32-58; pl. 31, figs. 64, 66.

It may be well to clarify in regard to the above synonymy that I have not examined material from sufficient appropriate localities to pass on the validity of Vargas' "*typicus*" and *stonei* as varieties of *punctipennis*. As presented by Vargas (1941) the ranges of these forms appear to be weirdly interlaced for geographic subspecies. If, like *perplexens*, they should be found to represent separate species, the ranges given by Vargas would appear to open the question as to which form corresponds better to Say's *punctipennis*. Freeborn (1949) predicted that a careful study of *punctipennis* throughout its range might show that several forms could be distinguished.

The species, *punctipennis* and *perplexens*, are easily distinguished on the qualitative characteristics of their respective ova as described and figured. Certain measurements of the ova will, alone, serve to separate these species in almost all cases (e.g. ratio of narrowest exposure of endochorion between floats to egg length), but in the series of ova measured there was at least slight overlap in all of the dimensions examined. Reliable characters for distinguishing the pupae have not been found. Fourth instar larvae and adults may not be distinguished with certainty as individuals, but marked statistical differences of certain characters have been demonstrated. Thus, while the identity of an individual egg can be recognized, an individual larva or adult must

be given a probable (or tentative) identification.

The status of *punctipennis* and *perplexens* as described above is exactly comparable to the situation in the *Anopheles maculipennis* complex in Europe in which different species are readily distinguished on characters of the eggs, while only statistical differences in the number of branches of the antepalmate hairs of abdominal segments IV and V characterize the larvae (Bates, 1939). An application of the statistical differences in the branching of these hairs was made by Bates (1941) in determining the members of the *maculipennis* complex represented in aggregations of larvae from various breeding areas in Albania. Utilization of the data in figure 3 to interpret the collections of larvae treated in figures 5 and 6 is a similar application of statistical criteria, in this case, for determining the probable constituency of natural populations of the *punctipennis* complex.

Comparison of the analyses in figure 5 with those in figure 3 clearly indicates that *perplexens* specimens predominate in the four aggregations of *punctipennis* complex larvae from limestone springs. The collection from a spring on Coolewahee Creek in Dougherty County consisted of only fifteen specimens and it appears probable that about half of these were *punctipennis*. By comparing the analyses given in figure 6 with those of figure 3 it is readily seen that the three collections of larvae from temporary waters constitute populations of *punctipennis*, at least in the main. It is not possible, of course, to conclude from the analyses given in figure 6 that a small number of *perplexens* larvae are not included in these collections (any more than is it possible to say that the analyses in figure 5 preclude the presence of *punctipennis* in the collections from limestone springs). But the fact that adult females captured in the environs of floodplain pools, etc. (when these are distant from limestone springs and "sink holes") deposit none but *punctipennis* ova, is strong evidence that *perplexens* rarely, if ever, invades such situations; furthermore, the adults taken in such localities regularly have the superficial appearance of *punctipennis*. On the other hand, *punctipennis* invades to some extent the habitats of *perplexens*. This is indicated both by the pilotaxy of larvae from limestone springs (figure 5) and by the fact that several groups of adult females collected in the environs of these springs have included a few specimens which deposited *punctipennis* ova.

In the same manner as above, the statistical criteria of figure 3 can be superimposed on the tabulation by Roth (1945) of the branching of hair 2 on abdominal segments IV and V of 1,821 larvae of "*A. punctipennis*" [*punctipennis complex*]. From this comparison it appears that less than 5% of Roth's larvae were *perplexens*. The comparison is not precise since Roth counted all branches of hair 2 irrespective of their point of origin and the analysis graphically presented in figure 3 does not include branches that originate beyond midlength of the hair.

Data on the relative portion of the wing length occupied by the subcostal spot (figure 4) indicate marked average differences between *punctipennis* and *perplexens* whether the specimens be reared or collected in the field. Wing lengths of comparably reared specimens are also greater in *punctipennis* than in *perplexens*, but the small series of wild egg-laying females of the two forms were found to have similar wing lengths on the average. Most of the egg-laying *punctipennis* females whose wings were measured were collected during a very dry season, and their maturation and emergence probably occurred in much receded pools in which there may have been considerable concentration of animal and plant wastes. This may have resulted in the production of stunted individuals. In any case, it seems probable that the data on wing lengths of egg-laying *punctipennis* are unintentionally biased, and that the average wing length of a less biased sample would be much greater. The greater average wing length of wild non-egg-laying females from *punctipennis* localities suggests this.

Roth (1945) reported the association of melanistic *punctipennis* [complex] adults with larvae having simple antepalmate hairs on abdominal segments IV and V from army installations at or near Paris, Tenn., and Swannanoa, N. C. These localities accordingly appear to be stations for *A. perplexens*, and clear pool-forming springs issuing from limestone strata, the habitat of *perplexens*, may be anticipated. Similarly, it may be predicted that there is a limestone spring in the vicinity of Camp Roosevelt at Mt. Gretna, Pa., where the type of *perplexens* was collected in 1906. In Lee County, Ga., specimens of *perplexens* have been collected by the writer both from limestone springs and from steep-sided lime sinkholes a few miles east of Leesburg, and the four specimens reported by Russell (1925) from this county which seem to correspond to this species were undoubtedly collected at one of these or a similar situation. The writer has also taken specimens at Magnolia Springs (a clear limestone spring) in Jenkins County, Ga., the locality from which Lawlor (1940) reported "smaller, darker specimens" [of the *punctipennis* complex]. Limestone springs are numerous in central Florida, and it is probably from these, and the spring runs that they form, that King, Bradley, and McNeel obtained their material which showed a preponderance of *perplexens* characters. The question should be raised as to whether or not *punctipennis* occurs at all in central Florida.

It is interesting that all the localities in Georgia from which *perplexens* has been collected were formerly highly malarious. These include the northwest corner of Baker County; the Kelg Creek system at Leary in Calhoun County;

Radium Springs and the Coolewahee Creek system near Albany and Pretoria, respectively, in Dougherty County; the Starksville Bridge area northeast of Leesburg in Lee County; at Leslie in the southwest corner of Sumter County; near Cordele and near the Flint River in Crisp County; and at Magnolia Springs in Jenkins County. This is probably no more than coincidental since *quadrimaculatus* and *crucians* have also been taken in all of these areas and *punctipennis* and *walkeri* are known from some of them. It is regrettable, however, that when malaria was of frequent occurrence in these localities, *perplexens* was not recognized as an entity, especially since in some parts of the world important vector species are sylvan and quite secretive in habit and return to the forest after feeding on man.

It may be assumed that studies of the life history, behavior, seasonal occurrence, geographic and ecological distribution, etc. reported for "*punctipennis*" apply in general to *punctipennis* as restricted in this paper, but in at least some cases the data have been obtained from study of mixed populations of *punctipennis* and *perplexens*. Data on blood feeding habits (or host preference), natural infection with *Plasmodia*, and efficiency as laboratory vectors of human malaria should be examined closely. Information as to the sources of specimens, when available, may be utilized to advantage in interpreting such data.

In the course of this study, a considerable amount of material was handled as individual lots of specimens representing in each case the progeny of only one female. It became increasingly apparent that there was much more morphological homogeneity among siblings than in mixed lots of material derived from a number of female parents. This phenomenon appeared to apply approximately equally to *punctipennis* and *perplexens*, and was observed in the size and proportions of ova, in the pilotaxy of the fourth stage larvae, and in the size and patterns of the wings of adults. Genetic factors are probably responsible for the homogeneity of individual batches of siblings and the striking differences between batches, but the conditions under which specimens were reared were not sufficiently uniform (some pans of larvae were more crowded than others, etc.) to preclude the possibility that the brood phenomena were environmentally determined. An environmental influence may be seen in the average size (based on wing lengths) of *punctipennis* adults reared in tap water and creek water, respectively. Further investigation under more controlled conditions is needed. Roth's work (1944, 1945, 1946) on the variation within species of mosquitoes emphasizes the importance of dealing with large representative samples to avoid obtaining a distorted picture of the morphology of a species; for the same reason, the brood effect mentioned above should especially be guarded against (material should be obtained from a number of sources).

The sympatric species, *perplexens* and *punctipennis*, pose a number of unanswered biological problems. Their geographical distribution should be investigated and their ecological relationships studied at various geographic points. As implied above, we may assume that there are few if any data on the blood feeding habits of *perplexens* or on the capacity of this species to transmit malaria. This pair of species appears to be ideal material for a study of geographic variation. The one species, *punctipennis*, is apparently very widespread and of almost ubiquitous occurrence, and isolated populations are probably at a minimum. On the other hand, *perplexens* appears to be highly restricted, at least in its ecological requirements, and each population is probably more or less isolated from the next. There should be much greater opportunity in *perplexens* than in *punctipennis* for the development of entirely different forms in these various discrete populations.

SUMMARY

Data are presented which indicate that *Anopheles perplexens* Ludlow, 1907 is a valid species, and it is, therefore, resurrected from the synonymy of *punctipennis* (Say) with which it has generally been confused. While extremely similar morphologically to *punctipennis* in almost all stages, the ova of *perplexens* are strikingly different, the differences as illustrated serving as the most reliable means of separating the two species. A comparison of the chaetotaxy of fourth stage larvae and pupae of *perplexens* and *punctipennis* is made. In fourth instars of *perplexens*, hair 2 on abdominal segments IV and V is predominantly single, but in *punctipennis* is generally branched. Adults of *punctipennis* are larger (longer wings) than *perplexens* on the average, and the light subcostal spot is shown to be relatively reduced in the average wing of *perplexens*. Reliable differences were not detected in a canvass of the mouth parts, forelegs, and hypopygia of representative adults of both sexes of the two species.

While *punctipennis* is widespread and appears to develop in a considerable variety of temporary and permanent waters, *perplexens* appears to be restricted mainly to limestone springs and the streams that they form. Recognition of the duality of the complex that has masqueraded as *punctipennis* raises certain problems as to the interpretation of various published data on "*punctipennis*," and points the way to further investigation of the ecological, genetic, and geographic relationships of these sibling species.

ACKNOWLEDGEMENTS

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CHAOBORINAE FROM TRINIDAD, B.W.I. (DIPTERA, CULICIDAE)[1]

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The Rockefeller Foundation (Trinidad Regional Virus Laboratory, Port-of-Spain, Trinidad, B.W.I.)

The material studied in this paper comprises collections made in Trinidad (B.W.I.) chiefly by the junior author. Eleven species of *Corethrella*, two of *Lutzomiops* and three of *Sayomyia* were obtained. Of the above mentioned species two are described as new. The allotypes of *C. blanda*, *C. downsi*, *C. flavitibia*, *C. jenningsi*, *C. tarsata and C. tripunctata* are here selected and described. In addition, the pupa and larva of *C. flavitibia*, *C. jenningsi*, *C. tarsata*, *L. juquiana* and *S. souzai* are described. Of the 16 species discussed, all but five (three *Corethrella* and two *Sayomyia*) are reported from Trinidad for the first time.

Valuable data were obtained regarding the aquatic breeding places of several species as well as the capture of adults.

Type material, unless otherwise indicated, is deposited in the collection of the Faculdade de Higiene e Saúde Pública da Universidade de São Paulo, Brazil.

The authors take pleasure in thanking Dr. W. G. Downs who aided in the collecting and Mr. E. B. Ferraz who did most of the drawings.

Corethrella ananacola Dyar 1926

Corethrella ananacola Dyar, 1926, Ins. Ins. Mens., 14: 150; Lane, 1953, Neotrop. Culicidae, 1: 83.
Corethrella inca Lane, 1939, Bol. Biol., (n.s.), 4: 103; Lane, 1942, Rev. Ent., 13: 113.

We have a male and a female.

Distribution.—Trinidad (B.W.I.), Arena Forest, San Rafael, 3/X/55 and 10/X/55 (T. H. G. Aitken, col.). Elsewhere the species is reported from Panama (type locality), Venezuela and Peru. (Lane, 1953).

Bionomics.—Both of the Trinidad specimens were collected at a light trap (white light) between 17:30 and 19:15 hours.

Corethrella blanda Dyar 1928

Corethrella blanda Dyar, 1928, Ent. News, 39: 79; Lane, 1953, Neotrop. Culicidae, 1: 81.

One female agreeing with descriptions is here selected as the allotype of this species.

Female.—Differs from the male by the following characters:—Palpus dark brown. Iridescent spot of sternopleuron extending to the upper portion of mesepimeron.

Allotype.—A female, registered in our collections under no. 11,428.

Allotype locality.—Trinidad (B.W.I.), Arena Forest, San Rafael, 10/X/1955 (T. H. G. Aitken col.).

Distribution.—Previously known only from the type locality of Las Sabānas, Panama (Lane, 1953).

Bionomics.—Two female specimens were collected at a light trap (white light) between 17:30 and 19:15 hours. Dyar (1928) reports the type material being reared from larvae from a grassy pool.

Discussion.—It is apparent from Dyar's description that the pupa of *blanda* is of a type similar to that of *Lutzomiops juquiana* as described later in this paper. Dr. Alan Stone confirms this impression after comparing our sketch with the type material in the United States National Museum.

Corethrella cardosoi Lane 1942

Corethrella (Corethrella) cardosoi Lane, 1942, Rev. Ent. 13: 118.
Corethrella cardosoi Lane, 1953, Neotrop. Culicidae, 1: 86.

We have noted that the hind tibia is darkened only at apex in the twelve specimens studied.

Distribution.—Trinidad (B.W.I.), Arena Forest, San Rafael, 7/II/55, 23/V/55, 15/VII/55, 22/VIII/55, and 3/X/55 (T. H. G. Aitken, col.). Elsewhere the species is reported from the States of São Paulo and Rio de Janeiro, Brazil (Lane, 1953).

Bionomics.—Twelve specimens collected at a light trap, 3 females from 17:30 to 19:15 hours and 9 females from 18:00 to 19:15 hours. On one occasion a red light was used.

Corethrella downsi Lane 1943

Corethrella (Corethrella) downsi Lane, 1943, Rev. Ent., 14: 164.
Corethrella downsi Lane, 1953, Neotrop. Culicidae, 1:77.

Quite a large series of specimens. A male has been selected as the allotype of this species.

Male.—Similar to female but antenna two and a half times as long as mesonotum and with more developed whorls.

Genitalia: (Fig. 1). Basistyle elongate, gradually tapering towards apex; basally a stout, differentiated and pointed seta; from basal to

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apical portion an irregular row of five slender, pointed setae. Dististyle nearly as long as basistyle, slender, with a long, slender seta basally. Other structures as in the figure.

Pupa.—The following characters are here added to the original description:—Two dark spots on sides of abdominal segment I. Cephalothorax darkened dorsally. Hairs A and B conspicuous,

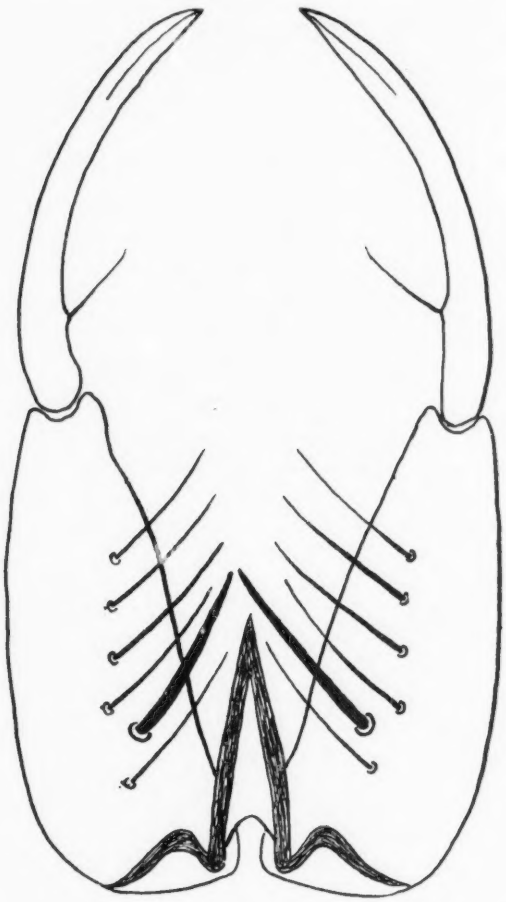

FIG. 1. *Corethrella downsi* Lane, 1943—Male genitalia (E. B. Ferraz, del.).

A on segments II through VII and B, III through VII robust and dark brown, contrasting with the general yellow color of abdomen. Hair B longer than segment on III and IV, subequal on V and shorter on VI and VII.

Larva.—With purplish markings on cephalothorax as well as on segments I, II and V. We add also the following characters to the original description:—Head with a conspicuous branched hair lateral to eye and bifurcate at about middle or beyond. Anal segment with set of three subdorsal setae, the dorsal pair double and three times length of third seta which is very robust; ventral tuft of four long double setae.

Allotype.—A male registered under no. 10,569.

Allotype locality.—Trinidad (B.W.I.), St. Patrick Estate, 3½ mile post, Arima Valley, elev. 800 feet. Reared from a larva collected in a bamboo pot, 27/III/54 (W. G. Downs, col.).

Distribution.—Trinidad (B.W.I.). Also De Gannes Village (Alta Gracia Trace), Siparia, 15/XI/54 (T. H. G. Aitken and W. G. Downs, col.); Charuma Forest, Tabaquite, 4/V/55 (T. H. G. Aitken, col.); Arena Forest, San Rafael, 7/II/55 (T. H. G. Aitken, col.); Aripo Caves, elev. 2,500 feet, 20/III/55 (W. G. Downs, col.); Mt. El Tucuche, elev. 2,100 and 3,000 feet, 27/III/55 (T. H. G. Aitken & W. G. Downs col.); Morne Bleu, elev. 1,900 feet, 9/IV/55 (T. H. G. Aitken & W. G. Downs, col.); and Valencia Old Road, Matura, 27/III/56 and 8/V/56 (T. H. G. Aitken, col.). Type locality, Montevideo, Sans Souci, Trinidad. Elsewhere the species is reported from Panama (Lane, 1953).

Bionomics.—The immature stages may lie quiescent below the water surface for long periods if disturbed. Specimens from the Aripo Caves lay at the surface with the abdominal segments curved down; the pupae were sluggish. The pupal period in the laboratory lasts about 5 to 7 days.

Corethrella downsi is the most eclectic species found in the present study, being taken in a variety of containers and from the lowlands to the tops of the Northern Range. In addition to bamboo pots, the aquatic stages have been found in water held by the floral bracts of *Heliconia elongata* Griggs (=*bihai* of West Indian authors) and the leaf axils of the following bromeliads: *Aechmea nudicaulis* (L.) Griseb., *A. mertensii* (Meyer) Schult., *Guzmania monostachia* (L.) Rusby ex Mez, *G. lingulata* (L.) Mez, *Gravisia aquilega* (Salisb.) Mez, *Vriesia procera* (Mart.) Wittm., *Vriesia splendens* var. *longibracteata* (Bak.) L. B. Sm., *Catopsis floribunda* (Brong.) L. B. Sm. and *Glomeropitcairnia erectiflora* Mez. Lane (1953) previously reports it from *Aechmea dichlamydea* var. *trinitensis* L. B. Smith.

On one occasion (7/II/55), a female *downsi* was taken in a light trap (white light) suspended six feet above the ground between 17:30 and 19:00 hours.

Corethrella flavitibia Lane 1939

Corethrella flavitibia Lane, 1939, Bol. Biol. (n.s.), 4: 392; Lane, 1953, Neotrop. Culicidae, 1: 85.

A male and female were reared and both the larval and pupal pelts mounted. The male is here selected as the allotype of this species.

Male.—Antenna with hairs longer than in female and forming whorls, the hairs slightly darkened apically.

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Corethrella (Corethrella) downsi Lane, 1943, Rev. Ent., 14: 164.
Corethrella downsi Lane, 1953, Neotrop. Culicidae, 1:77.

Quite a large series of specimens. A male has been selected as the allotype of this species.

Male.—Similar to female but antenna two and a half times as long as mesonotum and with more developed whorls.

Genitalia: (Fig. 1). Basistyle elongate, gradually tapering towards apex; basally a stout, differentiated and pointed seta; from basal to

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apical portion an irregular row of five slender, pointed setae. Dististyle nearly as long as basistyle, slender, with a long, slender seta basally. Other structures as in the figure.

Pupa.—The following characters are here added to the original description:—Two dark spots on sides of abdominal segment I. Cephalothorax darkened dorsally. Hairs A and B conspicuous,

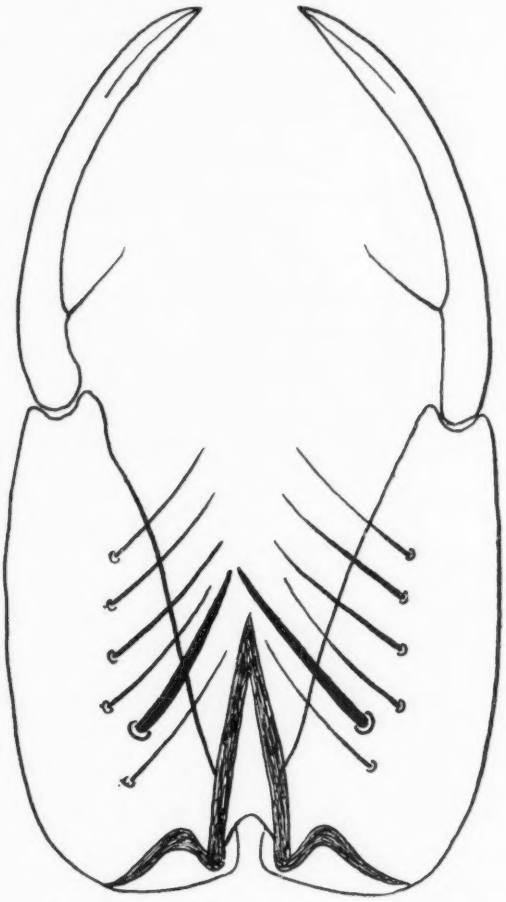

FIG. 1. *Corethrella downsi* Lane, 1943—Male genitalia (E. B. Ferraz, del.).

A on segments II through VII and B, III through VII robust and dark brown, contrasting with the general yellow color of abdomen. Hair B longer than segment on III and IV, subequal on V and shorter on VI and VII.

Larva.—With purplish markings on cephalothorax as well as on segments I, II and V. We add also the following characters to the original description:—Head with a conspicuous branched hair lateral to eye and bifurcate at about middle or beyond. Anal segment with set of three subdorsal setae, the dorsal pair double and three times length of third seta which is very robust; ventral tuft of four long double setae.

Allotype.—A male registered under no. 10,569.

Allotype locality.—Trinidad (B.W.I.), St. Patrick Estate, 3½ mile post, Arima Valley, elev. 800 feet. Reared from a larva collected in a bamboo pot, 27/III/54 (W. G. Downs, col.).

Distribution.—Trinidad (B.W.I.). Also De Gannes Village (Alta Gracia Trace), Siparia, 15/XI/54 (T. H. G. Aitken and W. G. Downs, col.); Charuma Forest, Tabaquite, 4/V/55 (T. H. G. Aitken, col.); Arena Forest, San Rafael, 7/II/55 (T. H. G. Aitken, col.); Aripo Caves, elev. 2,500 feet, 20/III/55 (W. G. Downs, col.); Mt. El Tucuche, elev. 2,100 and 3,000 feet, 27/III/55 (T. H. G. Aitken & W. G. Downs col.); Morne Bleu, elev. 1,900 feet, 9/IV/55 (T. H. G. Aitken & W. G. Downs, col.); and Valencia Old Road, Matura, 27/III/56 and 8/V/56 (T. H. G. Aitken, col.). Type locality, Montevideo, Sans Souci, Trinidad. Elsewhere the species is reported from Panama (Lane, 1953).

Bionomics.—The immature stages may lie quiescent below the water surface for long periods if disturbed. Specimens from the Aripo Caves lay at the surface with the abdominal segments curved down; the pupae were sluggish. The pupal period in the laboratory lasts about 5 to 7 days.

Corethrella downsi is the most eclectic species found in the present study, being taken in a variety of containers and from the lowlands to the tops of the Northern Range. In addition to bamboo pots, the aquatic stages have been found in water held by the floral bracts of *Heliconia elongata* Griggs (=*bihai* of West Indian authors) and the leaf axils of the following bromeliads: *Aechmea nudicaulis* (L.) Griseb., *A. mertensii* (Meyer) Schult., *Guzmania monostachia* (L.) Rusby ex Mez, *G. lingulata* (L.) Mez, *Gravisia aquilega* (Salisb.) Mez, *Vriesia procera* (Mart.) Wittm., *Vriesia splendens* var. *longibracteata* (Bak.) L. B. Sm., *Catopsis floribunda* (Brong.) L. B. Sm. and *Glomeropitcairnia erectiflora* Mez. Lane (1953) previously reports it from *Aechmea dichlamydea* var. *trinitensis* L. B. Smith.

On one occasion (7/II/55), a female *downsi* was taken in a light trap (white light) suspended six feet above the ground between 17:30 and 19:00 hours.

Corethrella flavitibia Lane 1939

Corethrella flavitibia Lane, 1939, Bol. Biol. (n.s.), 4: 392; Lane, 1953, Neotrop. Culicidae, 1: 85.

A male and female were reared and both the larval and pupal pelts mounted. The male is here selected as the allotype of this species.

Male.—Antenna with hairs longer than in female and forming whorls, the hairs slightly darkened apically.

Genitalia: (Fig. 2). Basistyle about twice as long as wide bearing basally a stout strong seta which is blunt at apex; near this seta a similar one which is not so stout and pointed at apex; four long, slender, pointed setae in an irregular line from middle to apex. Dististyle about as long as basistyle, basally a long slender seta on internal margin. Other structures as in the figure.

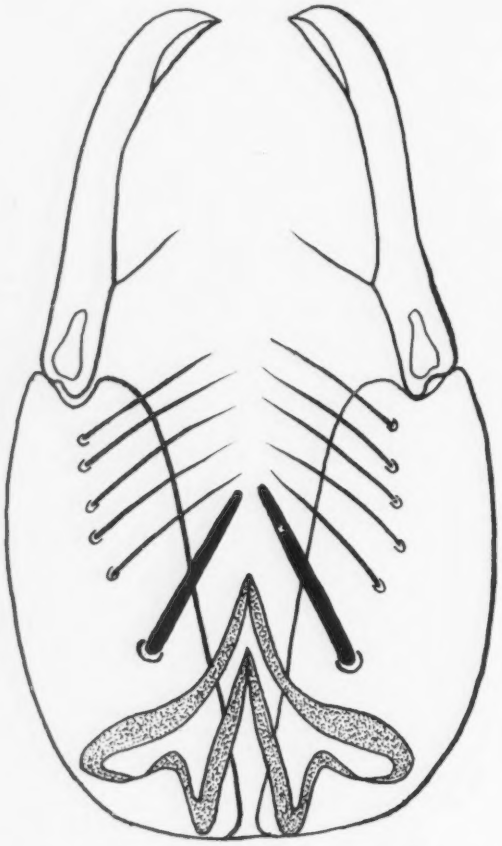

FIG. 2. *Corethrella flavitibia* Lane, 1939—Male genitalia (E. B. Ferraz, del.).

Pupa (Fig. 3).—Tube small, ovate, reticulate, basally expanded, length twice greatest width, the opening rounded and apical. Cephalothorax with dorsal portion darker, a long hair near tube, the other hairs very small.

Abdomen: Segments I and II slightly darker, setae small; III through VI quite darkened in the middle, on V and VI this darkening invades the sides posteriorly; VII slightly darker at base; segment VIII not marked. Hair B as long as segment in III through V, very short on VI and VII. Hair A short but conspicuous, on IV, V and VI; on III and IV there is a supplementary short lateral hair. Paddle one and a half times as long as segment VIII, ending in a spine and a hair, another long hair on side externally.

Larva.—Head (Fig. 4) subtriangular. Antenna about half as long as head, ending in three long, curved setae (two of which are subequal) and a feathered spicule. Row of lateroposterior teeth of about twelve subequal elements. Chaetotaxy as in the figure. Mandible and maxilla as in the figure.

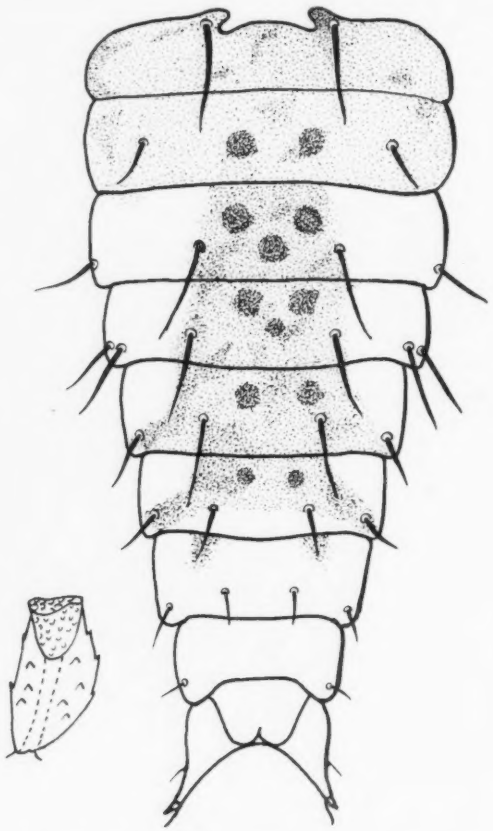

FIG. 3. *Corethrella flavitibia* Lane, 1939—Pupal respiratory tube and abdomen (T. H. G. Aitken, del.).

Body nude with long, single hairs. Siphon length about one and a half times its basal width; long terminal hooks, posterior pair shorter. Anal segment as long as siphon, apically with a set of three subdorsal setae, the upper pair double and much longer than single third seta; ventral tuft formed of four double setae.

Allotype.—A male, with pupal and larval pelts, registered under no. 10,563.

Allotype locality.—Trinidad (B.W.I.), Arima-

Blanchisseuse Road, 9 mile post, elev. 1,600 feet. Reared from a larva collected in *Aechmea nudicaulis* (L.) Griseb., 11/XII/53 (W. G. Downs, col.).

Distribution.—Trinidad (B.W.I.). Also Mt. El Tucuche, elev. 3,000 feet, 27/III/55 (T. H. G. Aitken and W. G. Downs, col.). Elsewhere the species is known only from the holotype from Juquiá, São Paulo, Brazil.

Bionomics.—The immature stages spend much of their time below the water surface in a more or less quiescent state, the larvae being stimulated into action when prey comes in contact with them. In addition to the allotype, the species was encountered on one other occasion, breeding in another bromeliad, *Vriesia johnstonei* Mez, near the summit of El Tucuche. The pupa of this specimen was very sluggish, but exhibited undulating movements when stimulated. Eventually it would come to rest at the surface. The pupal period in the laboratory lasted four days; a female was reared. Note: Mean minimum and maximum outside temperatures for April varied between 71° F and 87° F.

FIG. 4. *Corethrella flavitibia* Lane, 1939—Larval head (E. B. Ferraz, del.).

Corethrella jenningsi Lane 1942

Corethrella (*Corethrella*) *jenningsi* Lane, 1942, Rev. Ent., 13: 106; Lane, 1953, Neotrop. Culicidae, 1: 75.

Female.—The general coloration can be of a lighter or darker tinge, especially on the thorax. Femora are dark at base and yellowish at apex. Hind tibia has a definite dark brown apical band in some specimens. The abdomen tends more to a reddish yellow.

Male.—Flagellum sparsely plumose but hairs much longer than in the female and forming whorls. Femora dark at base and yellowish at apex.

Genitalia: (Fig. 5). Light yellow. Basistyle elongate, about three times the basal width, external margin with long setae, the internal with short ones; a stout blunt basal seta, above it another differentiated seta and yet another one just below it, both these setae slender and pointed; distally four or five irregularly placed long, prominent setae. Dististyle three fourths the length of basistyle, a prominent seta on its basal fourth. Other structures as in the figure.

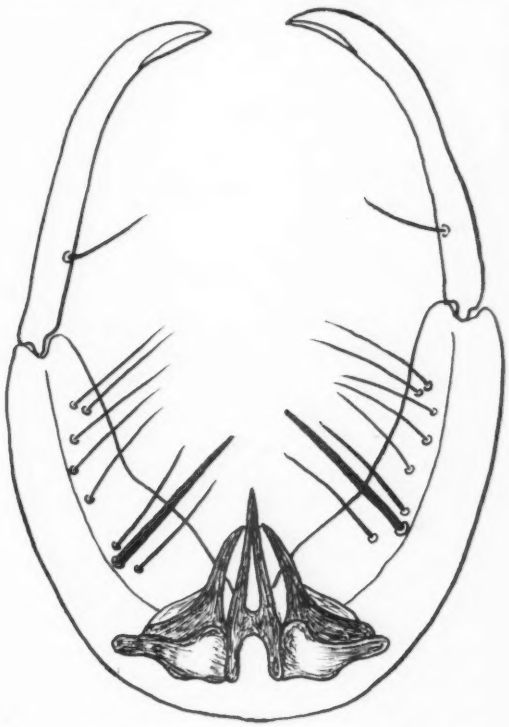

FIG. 5. *Corethrella jenningsi* Lane, 1942—Male genitalia (E. B. Ferraz, del.).

Pupa (Fig. 6).—Generally yellow except for brown area on dorsum of cephalothorax between tubes and extending posteriorly between metanotum to first abdominal segment. Tube elongate, three times apical width, spinose externally (reticulate internally), broadened apically and with a lateral notch. Dorsal prothoracic hairs strong, stout and single, and arising from conspicuous chitinized tubercle; a conspicuous pair

arising side by side, anterior to each tube from prominent tubercle.

Abdomen with the margins of segments serrate; hair B stout, one and a half times as long as respective segments on III, IV and V; on VI as long as segment and not as stout as the other B hairs. Hair A long, stout and present on segments II through VII; a supplementary hair, as long as A present on V through VII. Paddle twice as long as VIII; apex with a blunt spine and a slender hair, another slender hair on apical third externally.

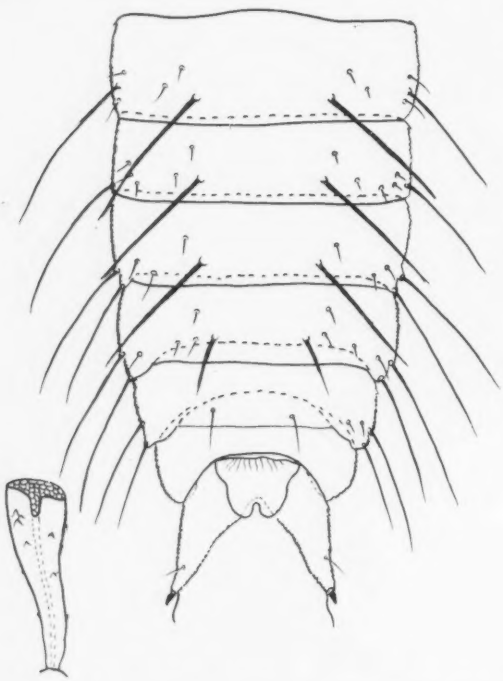

FIG. 6. *Corethrella jenningsi* Lane, 1942—Pupal respiratory tube and abdominal segments III–VIII (E. B. Ferraz, del.).

Larva.—Head (Fig. 7) subquadrate, quite broader than long. Antenna one third shorter than length of head; ending in three long, curved setae (one longer than other two) and a feathered spicule. About 16 posterolateral teeth, which are mostly long and blunt. Hairs as in figure, a long-branched hair (*bifurcate from base*) anterior to posterolateral teeth. Mandible as in figure. Mentum with 11 to 13 teeth with three conspicuous teeth in middle, separated by smaller ones.

Body nude, with long, single setae, chiefly on the sides. Eighth segment with posterior set of four conspicuous setae arising from prominent tubercles, two setae stronger, longer than others and single, remaining two shorter, more slender, and bifurcate. Siphon one and a half times as long as basal width, terminal hooks long, the posterior pair half as long as others and less chitinized; a branched seta arising near base of siphon. Anal segment considerably shorter than siphon and bearing a set of three subdorsal setae, two of these are long and double, the third shorter and single; ventral tuft of four longer, double setae.

Allotype.—A male with corresponding larval and pupal pelts, registered under no. 10,564.

Allotype locality.—Trinidad (B.W.I.), St. Patrick Estate, 3½ mile post, Arima Valley, elev. 800 feet. Reared from a larva collected in the leaf axil of *Xanthosoma* sp., 7/I/54 (W. G. Downs, col.).

Distribution.—Trinidad (B.W.I.), St. Patrick Estate, Arima Valley, 22/XII/53, 22/I/54,

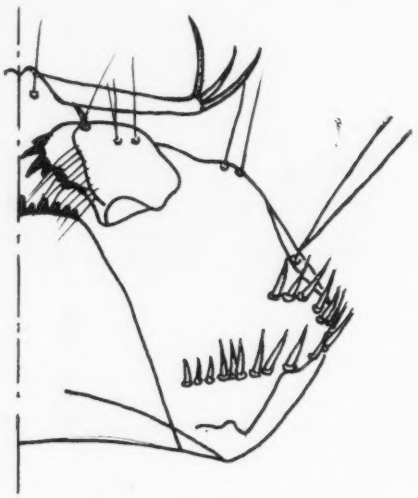

FIG. 7. *Corethrella jenningsi* Lane, 1942—Larval head (E. B. Ferraz, del.).

5/II/54, 5/III/54 (W. G. Downs, col.), 19/X/54, 22/XII/54, 24/III/55, 16/VI/55 and 27/VII/55 (T. H. G. Aitken, col.); Cumaca, elev. 1,200 feet, 27/V/54 (T. H. G. Aitken and W. G. Downs, col.); La Laja Trace, Arima Valley (elev. 2,100 feet), 5/VI/55 (T. H. G. Aitken, col.); and Arena Forest, San Rafael 27/I/54, (W. G. Downs, col.). Elsewhere the species is known only from the type locality in the Panama Canal Zone.

Bionomics.—In the laboratory, the immature stages appear to spend most of their time motionless at the bottom of the container. The larvae readily feed on young mosquito wigglers. The species has been collected on a number of occasions from the water in the leaf axils of *Xanthosoma* sp.; it was also taken from the leaf axils and floral bracts of *Calathea trinitensis* Britton and the leaf axils of *Cyclanthus bipartitus* Poit. as well as from a bamboo pot. On one occasion a female

came to the Arena Forest light trap between 18:10 and 19:00 hours.

Corethrella melanica n. sp.

Female.—Head: Mouth parts yellowish. Palpus whitish yellow, segments II and IV longer than others and subequal. Clypeus blackish, lateral margins darker, basally with two long, approximated setae. Antenna: scape small and brown; torus blackish with small golden scales above and a row of small, short, black setae; flagellum short, pilose, two and a half times as long as mesonotum, segments and setae blackish. Occiput blackish with whitish scales and long, black marginal setae, those on vertex long, proclinate and golden.

Thorax with blackish integument. Pronotal lobe blackish with pruinose bloom. Mesonotum with a pattern of whitish pruinose markings; acrostichal and dorsocentral setae long and black, a few golden shorter ones, other setae long and black; covered with short, sparse golden scales. Scutellum blackish with ten long, black marginal setae and shorter golden hairs and scales. Postnotum blackish brown, lighter on the sides. Pleura blackish, posterior sclerites darkest.

Wing: Basal two thirds predominantly dark scaled, the apical third contrastingly covered with dirty white scales. A somewhat indistinct narrow whitish fenestration on black portion. The distal black band covers all veins from junction of 1st vein to posterior fringe which is also blackish. The mesial fenestration of whitish scales includes a small portion of costal and subcostal veins, 1st vein, junction of 2nd and 3rd veins, 4th vein, fork of 5th and apex of 6th vein.[2] The rest is darkened to base with a faint indication of another more basal fenestration. Fringe darkened. Halter with pale stem and blackish knob.

Legs: Coxae blackish. Femora blackish, hind pair with distal third yellowish. Knees golden scaled. Tibiae blackish but golden apically, the hind pair with a broad golden stripe over whole inner surface except base and apex. Tarsi with distinct apical golden markings, basal portions blackish on external surface and golden scaled internally.

Abdomen blackish with dense yellowish hairs apically on tergites and giving the effect of bands. Sternites blackish.

Male.—Similar to female. Antenna with hairs longer and forming whorls, apparently a little shorter.

Wing with predominantly yellow scales and the following dark markings. A stripe from junction of costa with 1st vein and crossing the wing. At base a large dark mark from costa to 6th vein. Between these spots a small median one from apex of stem of 2nd to apex of stem of 5th vein.

Genitalia: (Fig. 8). Basistyle elongate, over twice greatest width; a basal stout, pointed seta and from near base toward apex an irregular row of five prominent setae (long, slender and pointed). Dististyle nearly as long as basistyle, a strong seta on basal third. Other structures as in the figure.

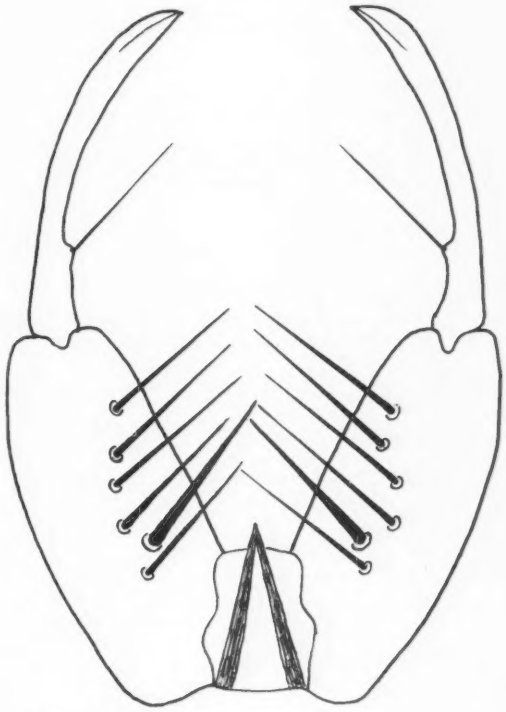

FIG. 8. *Corethrella melanica* n. sp. Male genitalia (E. B. Ferraz, del.).

Pupa (Fig. 9).—The living pupa is uniformly dark colored. Tube reticulate, ovate, somewhat attenuated apically, length about 2.5 times greatest width, covered with overlapping scales externally; apical opening large.

Abdomen with prominent hair on anterior median margin of segment I. Hair B on segments II through VI robust, nearly the length of respective segments. Hair A short, inconspicuous, most prominent on segments VII and VIII. Paddle about 1.5 times length of segment VIII, a pointed spine and a seta at apex and a slender one on external border.

Larva.—Head (Fig. 10) subquadrate. Antenna about one-third length of head, ending in three curved setae (two long and one shorter) and a

[2] A fair amount of variation exists in the wing markings.

short, feathered spicule. Row of lateroposterior teeth of 12 to 13 subequal elements. Mentum with 12 teeth. Mandible with eight strongly chitinized teeth, one much stouter than rest, and a number of accessory spines. Siphon and anal segment subequal in length, the former with long terminal hooks, the posterior pair shorter. Anal segment with a set of three subdorsal setae, the dorsal pair double and three times length of third single seta; ventral tuft of four long, double setae, one-third longer than subdorsal tuft.

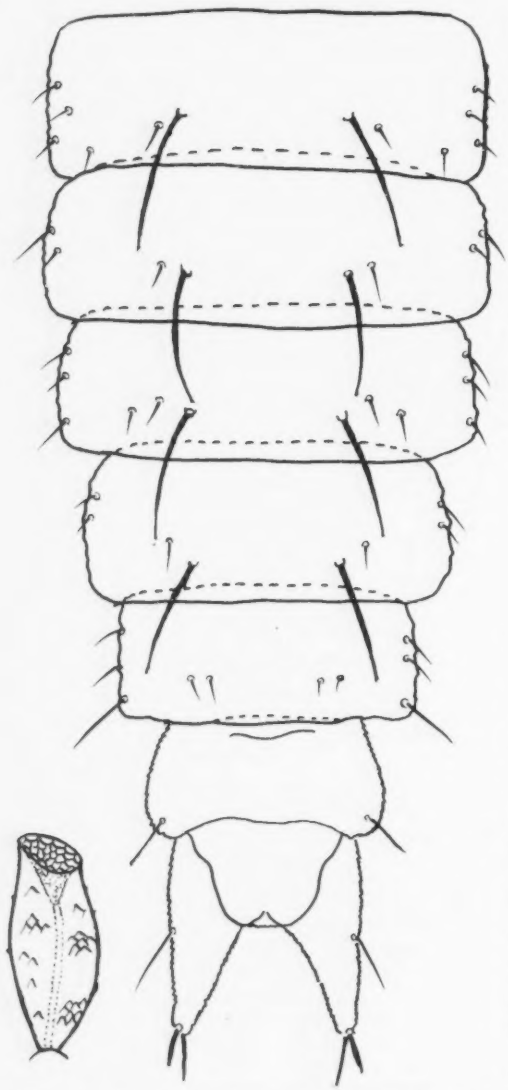

FIG. 9. *Corethrella melanica* n. sp. Pupal respiratory tube and abdominal segments III–VIII (E. B. Ferraz, del.).

Types.—Holotype female registered under no. 10,556; allotype male, registered under no. 10,561. Paratypes, 15 females and 7 males. Three male and 6 female paratypes registered under nos. 10,557 to 10,560, 10,562 and 10,944 to 10,947. Three female and 1 male paratypes to be sent to the British Museum (Natural History), London and 3 male and 6 female paratypes to the United States National Museum, Washington, D. C.

Type locality.—Trinidad (B.W.I.), St. Patrick Estate, 3½ mile post, Arima Valley, elev. 800 feet. Reared from larvae collected in bamboo pots, 6/II/54, holotype; 20/III/54, allotype (W. G. Downs, col.).

Distribution.—Trinidad (B.W.I.), St. Patrick Estate, Arima Valley, 2/II/54, 6/II/54, 23/II/54, 27/II/54, 29/II/54, 6/III/54, 20/III/54, (W. G. Downs, col.), 11/XII/54, and 16/IV/55 (T. H. G. Aitken, col.); Charuma Forest, Tabaquite,

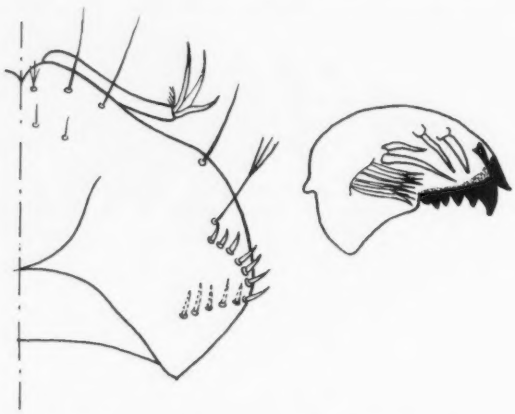

FIG. 10. *Corethrella melanica* n. sp. Larval head and mandible (T. H. G. Aitken, del.).

13/I/55, 24/II/55 and 6/IV/55 (T. H. G. Aitken, col.); Arena Forest, San Rafael, 3/X/55 (T. H. G. Aitken, col.); Macqueripe and Tucker Valley, United States Naval Station, 20/X/55 and 4/I/56 (T. H. G. Aitken, col.).

Bionomics.—The larvae and pupae spend much of their time in a more or less quiescent state at the bottom of the container or else hanging vertically from the surface film. The pupal period lasts from four to seven days in the laboratory. The immature stages have been taken in water-holding bamboo pots as well as once from a tree hole. Adults came to light traps on two occasions, between 18:00 and 19:15 hours in the Arena Forest and between 19:00 and 07:00 hours at Macqueripe.

Discussion.—This is the first time in which this type of sexual dimorphism has been encountered in a species of this genus; no other species has shown variation in the wing markings.

In the female, the larger size, yellowish palpus,

darker general coloration, more distinct and broader tarsal and tibial markings, wing with its contrasting coloration of basal dark and apical whitish scales separate this species from *C. travassosi* which it more closely resembles. In our key (Lane, 1953), *C. melanica* should be entered in dichotomy 5 and the above-mentioned characters which separate it from *C. travassosi* noted. The males, with different wing markings would fall in the above-mentioned key with *C. striata* from which they can be separated by the yellowish palpus and third dark spot, in the middle of wing.

Corethrella selvicola Lane 1939

Corethrella selvicola Lane, 1939, Bol. Biol. (n.s.), 4: 391; Lane, 1953, Neotrop. Culicidae, 1: 87.
Corethrella (Corethrella) selvicola Lane, 1942, Rev. Ent., 13: 119.

More than thirty specimens which agree with the types.

Distribution.—Trinidad (B.W.I.), Arena Forest, San Rafael, 9/III/54 (W. G. Downs, col.), 12/IV/55, 10/X/55 and 17/X/55 (T. H. G. Aitken, col.). Port-of-Spain is a previous locality record cited by Lane (1953). Elsewhere the species is known from Brazil (States of São Paulo, Rio de Janeiro, Bahia and Espirito Santo), British Guiana and Panama (Lane 1953).

Bionomics.—Only adults of *selvicola* have been taken by us and these came from light trap collections between 17:00 and 19:15 hours. On one occasion a red light was used.

Corethrella similans, n. sp.

Female.—Head: Mouth parts and palpus yellowish. Clypeus brown. Antenna with large blackish torus bearing verticils of golden hairs, flagellum three and a half times as long as mesonotum, with sparse verticils of longish brownish hairs on segments. Occiput blackish with white pruinosity and rows of black ocular setae.

Thorax: Pronotal lobe blackish with white pruinosity. Mesonotum blackish ornamented with a white pruinose pattern; acrostichal, dorsocentral and remaining setae short and black. Scutellum blackish with about eight marginal setae. Postnotum blackish, the sides lighter. Pleura blackish but upper mesepimeron white pruinose as well as mesial portion of mesepimeron and sternopleuron.

Legs yellowish, with a darkening of integument at extreme base of femora; dorsal surface of femora and fore- and mid-tibia with brown hairs. Tarsi with brown hairs on external surface and distinct whitish apical rings, inner surface yellowish, the rings indistinct.

Wing yellow scaled, the extreme base with darker scales. Halter whitish except for the base of knob which has small brown mark.

Abdomen blackish with sparse yellowish hairs.

Male—pupa and larva.—Unknown.

Types.—Holotype female, paratypes three females. Holotype and one paratype registered under nos. 11,429 and 11,430. Two remaining paratypes to be sent to the United States National Museum, Washington, D.C.

Type locality.—Trinidad (B.W.I.), Tucker Valley, U. S. Naval Station, 20/X/55 and one paratype from Arena Forest, San Rafael, 3/X/55 (T. H. G. Aitken, col.).

Bionomics.—The adults were collected at white light traps, the one female from Arena Forest between 18:00 and 19:15 hours, and the remainder from the Naval Station overnight between 19:00 and 07:00 hours.

Discussion.—This species has been named *similans* because of its very close resemblance to *Lutzomiops juquiana* (Lane, 1939). The chief difference lies in the tarsi which are distinctly banded in *similans* and are unicolorous in *juquiana*. Likewise the halter knob in the latter species is blackish, whereas the entire halter is almost completely pale in *similans*.

Corethrella tarsata Lane 1942

Corethrella (Corethrella) Lane, 1942, Rev. Ent., 13: 102; Lane, 1953, Neotrop. Culicidae, 1: 71.

We have selected as the allotype of this species one of three females with corresponding pupal and larval pelts.

Female.—Differs from the male by the following characters:—Antenna two and a half times the length of mesonotum with long filiform hairs. Scutellum yellowish, darker at sides, mesially with a longitudinal dark brown line. Wing scales with golden tinge. Hind femur with more than distal half yellowish. Tarsi with rings incomplete. Abdomen densely covered with long, golden hairs.

Pupa (Fig. 11).—Tube small, ovate, length twice greatest width, expanded at apex, opening large. Cephalothorax darker dorsally, the hairs small and simple.

Abdomen somewhat darker in the middle; all hairs simple. Hair A developed on segments II and V through VII. Hair B developed on segment III through VII but progressively shorter towards terminal segments. Segments with small posterolateral protuberances. Paddle nearly triangular, the hairs as in figure.

Larva.—Head (Fig. 12) triangular. Antenna slightly less than half the length of head, with three apical setae, two of these slightly longer than half the length of antenna. Lateroposterior setae three small and nine larger ones in an irregular row. Mandibles with four larger and two smaller teeth, the external margin spiculose. Mentum with thirteen teeth, the mesial one largest.

Body nude, hairs long and simple or multiple. Siphon with two pairs of long *curved* hooks, the posterior pair one third shorter. Anal segment

as long as siphon, apically with a set of three subdorsal setae, the upper pair double and much longer than single third seta; ventral tuft formed of four double setae.

Allotype.—A female together with pupal and larval pelts, registered under no. 3,401.

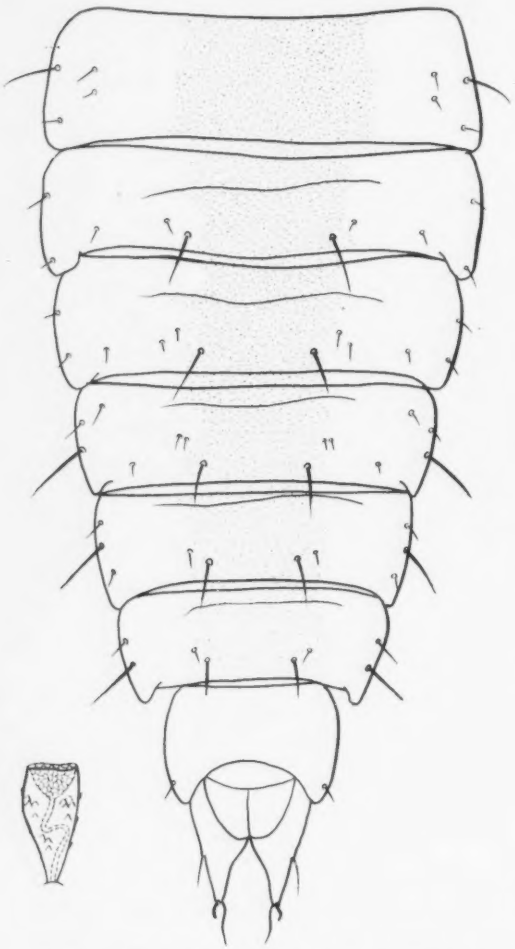

FIG. 11. *Corethrella tarsata* Lane, 1942—Pupal respiratory tube and abdominal segments II–VIII (E. B. Ferraz, del.).

Allotype locality.—Trinidad (B.W.I.)—Charuma Forest, Tabaquite, elev. 175 feet. Reared from a larva collected in the bromeliad *Aechmea nudicaulis* (L.) Griseb., 6/IV/55 (T. H. G. Aitken, col.).

Distribution.—Trinidad (B.W.I.), in addition to the above-mentioned April collection from Charuma Forest, a larva (subsequently reared out) was taken from the same plant on 24/II/55 (T. H. G. Aitken, col.), also Valencia Old Road, Matura, 16/V/56, reared ex *Gravisia aquilega* (Salisb.) (T. H. G. Aitken, col.). The species was previously reported from Cumuto (Lane, 1953). Elsewhere, *tarsata* is known only from the Brazilian type locality of Camassari, State of Bahia.

Bionomics.—The more or less quiescent immature stages are found in bromeliads and remain below the water surface much of the time. On one occasion, a *tarsata* larva was observed catching a fourth instar *Wyeomyia* larva which it devoured, all but the terminal two abdominal segments. Unfortunately this larva subsequently perished, but it was a large specimen and others which have completed their development, have produced adults larger than the usual *Corethrella* species.

FIG. 12. *Corethrella tarsata* Lane, 1942—Larval head (E. B. Ferraz, del.).

Corethrella tripunctata Lane 1942

Corethrella (*Corethrella*) *tripunctata* Lane, 1942, Rev. Ent. 13: 120; Lane, 1953, Neotrop. Culicidae, 1: 89.

The description of this species rests on a unique male specimen from Puerto Rico. A large series of Trinidadian females forms the basis of the present report and one was chosen as the allotype of the species.

Differs from the male by the following characters:—Antennal flagellum two and a half times as long as mesonotum. Pleura blackish brown. Legs with dark brown integument which becomes

lighter on the tarsi. Hind tibia paler in the middle (distinctly yellow on inner aspect), dark brown after base and before apex. Tarsi with very narrow (inconspicuous) whitish rings. Wing with mesial dark mark on band from costa to upper branch of 5th vein; apical dark spot reaching both branches of 2nd vein; basal spot extending in arc from costa to 4th vein; faint spots on posterior fringe of wing at apex of 4th vein, lower branch of 5th vein and near 6th vein. Scales of wing otherwise yellowish. Halter with white stem, the knob yellowish.

Allotype.—A female registered under no. 11,431.

Allotype locality.—Trinidad (B.W.I.), Tucker Valley, United States Naval Station, 20/X/55 (T. H. G. Aitken, col.).

Distribution.—Trinidad (B.W.I.), additional female specimens from the Naval Station include the following: Tucker Valley 20/X/55 (22 specimens), 27/X/55 (48), 1/XI/55 (4), 2/XI/55 (8), 3/XI/55 (4), 4/XI/55 (1), 9/XI/55 (6) and 11/I/56 (1); Chaguaramas (Grandwood), 27/X/55 (1); and Macqueripe 27/X/55 (2), 9/XI/55 (2) (T. H. G. Aitken, col.); Arena Forest, San Rafael 3/X/55 (T.H.G. Aitken, col.). The species was previously known only from the male type from Carolina, Puerto Rico (Lane, 1953).

Bionomics.—All Trinidadian specimens have been collected at light traps, the Arena Forest specimen between 18:00 and 19:15 hours and the Naval Station specimens between 19:00 and 07:00 hours. Lane (1953) records crab holes as a source of larvae in Puerto Rico.

Discussion.—Female *tripunctata* may be separated from *C. kummi* as follows: hind tibia of the former dark brown but paler in the middle, the inner aspect being largely yellowish; wing not peppered, the spots not much detached; and the yellow-knobbed halter.

Lutzomiops amazonica (Lane) 1939

Corethrella amazonica Lane, 1939, Bol. Biol. (n.s.), 4: 110.
Corethrella (Lutzomiops) amazonica Lane, 1942, Rev. Ent., 13: 131.
Lutzomiops amazonica (Lane), 1953, Neotrop. Culicidae, 1: 99.
Corethrella (Lutzomiops) coutinhoi Lane, 1942, Rev. Ent., 13: 131.

We have a male and 41 females. They are darker than Brazilian specimens and show slight differences.

Distribution.—Trinidad (B.W.I.), Arena Forest, San Rafael, 3/II/54 (W. G. Downs, col.), 23/II/55 (T. H. G. Aitken, col.); and United States Naval Station, Macqueripe, 20/X/55 (6 specimens), 27/X/55 (1), 31/X/55 (4); Chaguaramas (Grandwood), 27/X/55 (24), 9/XI/55 (1), and Tucker Valley, 27/X/55 (4). Elsewhere the species is known from the States of Amazonas, São Paulo and Espirito Santo, Brazil (Lane 1953), Costa Rica (Lane 1942) and Tocumen, Panama, 1955 (F. S. Blanton, col.).

Bionomics.—All of our material has come from light traps, that from Arena Forest being taken between 17:30 and 19:00 hours (once with a red light) and the Naval Station material between 19:00 and 07:00 hours. The larval habitat is unknown.

Lutzomiops juquiana (Lane) 1939

Corethrella juquiana Lane, 1939, Bol. Biol. (n.s.), 4: 109, 388.
Corethrella (Lutzomiops) juquiana Lane, 1942, Rev. Ent., 13: 131.
Lutzomiops juquiana (Lane), 1953, Neotrop. Culicidae, 1: 99.

A female with corresponding larval and pupal pelts, which are described below as well as two other females (one with pupal pelt). It has been noted that our specimens have black-knobbed halteres.

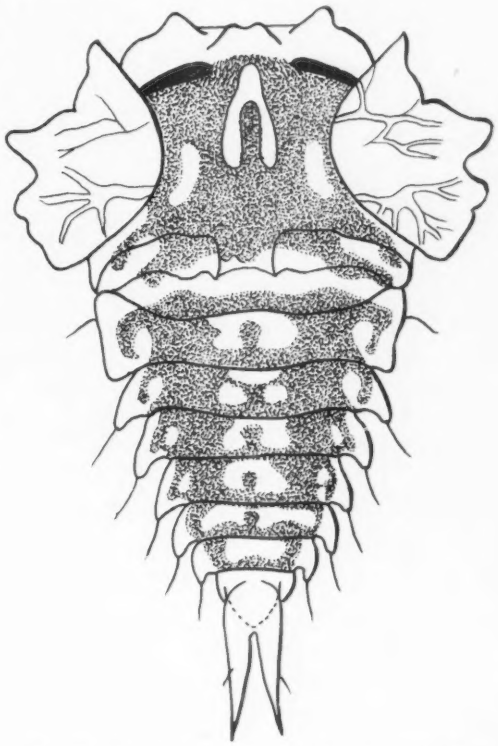

FIG. 13. *Lutzomiops juquiana* (Lane), 1939—Pupa drawn from life to show resting position, respiratory "epaulets" and markings (T. H. G. Aitken, del.).

Pupa.—The living pupa (Fig. 13) has blackish markings over a yellow-brown background. "Tube" highly modified in form of respiratory "epaulets," large convex lateral protuberances or "wings" with irregular contour; tracheoles visible in these membranous structures. Cephalothorax of abandoned pelt unicolorous, setae more or less long and simple.

Abdomen unicolorous (Fig. 14). Segments I and II average but III through VII with conspicuous postero-lateral protuberances which are serrate on margins and have hair A inserted at their apices. Hair A present on segments II through VIII but decreasing in size on terminal ones. Hair B small and progressively shorter on terminal segments, present only on III through VI. Segment VIII somewhat rounded. Paddles fused at base, quite long, ending in a filament with a stout blunt apical seta.

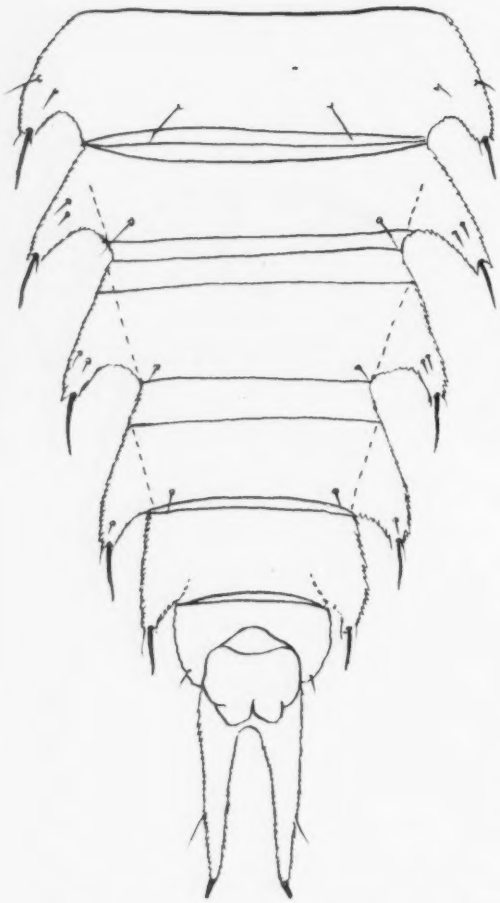

FIG. 14. *Lutzomiops juquiana* (Lane), 1939—Pupal abdominal segments III–VIII (E. B. Ferraz, del.).

Larva.—Head (Fig. 15) triangular. Antenna considerably shorter than length of head, ending in three setae half as long as antenna. Twelve lateroposterior setae in a row, some smaller than others. Mandible (Fig. 16) with seven apical teeth as in figure. Plate of mentum with eleven teeth, mesial one largest.

Body nude with numerous long setae and six characteristic intersegmental plates, two large and four small, similar to those in *C. downsi*. Siphon long, two and a half times greatest width, hooks curved and large. Anal segment apparently covered by the plate; dorsal hairs 2+2; ventral hairs in five double pairs. Segment VIII sclerotized and with long posterior setae.

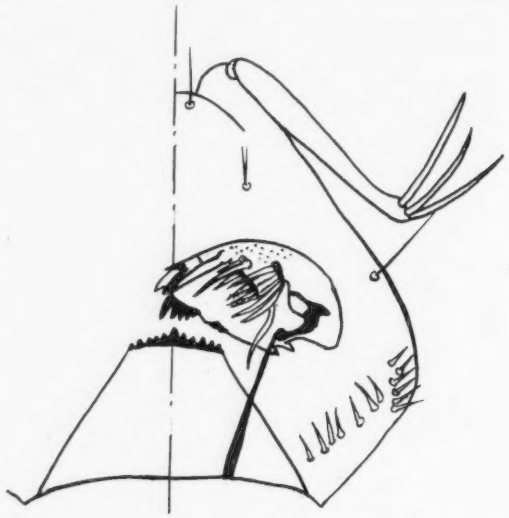

FIG. 15. *Lutzomiops juquiana* (Lane), 1939—Larval head (E. B. Ferraz, del.).

FIG. 16. *Lutzomiops juquiana* (Lane), 1939—Larval mandible (E. B. Ferraz, del.).

Distribution.—Trinidad (B.W.I.) United States Naval Station, Tucker Valley 9/II/55 and 1/II/56 and Macqueripe, 26/I/56 (T. H. G. Aitken, col.).

Bionomics.—The larva appears to prefer remaining at the water surface; if disturbed, it will quickly return to its former position. The pupa also prefers the surface where it lies *motionless* with the abdomen outstretched in a horizontal plane; this is in contradistinction to the resting pupa of *Corethrella*, which curves the abdominal segments down mosquito-fashion. The hydrophobic character of the *juquiana* respiratory "epaulets" apparently prevents the pupa from submerging. The immature stages have been found in a drain and a ground pool.

On one occasion a female was taken in a light trap operated between 19:00 and 07:00 hours.

Discussion.—Originally it was thought that this most interesting pupal type was unique or possibly of generic significance; however it appears that the pupa of *Corethrella blanda*, as originally described by Dyar, is of a similar type.

Sayomyia australis (Shannon & Del Ponte) 1927

Chaoborus australis Shannon & Del Ponte, 1927, Rev. Inst. Bact. Buenos Aires, 5: 99.
Chaoborus (Sayomyia) australis, Lane, 1942, Rev. Ent., 13: 143.
Sayomyia australis, Lane, 1953, Neotrop. Culicidae, 1: 103.

Two specimens which show very faint dots between the three mesonotal stripes.

Distribution.—Trinidad (B.W.I.), Arena Forest, San Rafael, 9/III/54 (W. G. Downs, col.) and 3/X/55 (T. H. G. Aitken, col). Known elsewhere from Argentina (type locality) and the Brazilian States of Rio de Janeiro, Minas Gerais and Matto Grosso.

Bionomics.—Adults were captured by us at a white light trap between 17:45 and 19:15 hours.

Sayomyia brasiliensis (Theobald) 1901

Corethra brasiliensis Theobald, 1901, Mon. Culicidae, 2: 302.
Chaoborus (Sayomyia) brasiliensis Lane, 1942, Rev. Ent., 13: 141.
Sayomyia brasiliensis, Lane, 1953, Neotrop. Culicidae, 1: 101.
Chaoborus festivus Dyar & Shannon, 1924, Ins. Ins. Mens., 12: 215.
Chaoborus antunesi Lane, 1939, Bol. Biol. (n.s.), 4: 392.

Five specimens which agree with descriptions.

Distribution.—Trinidad (B.W.I.), Arena Forest, San Rafael 15/III/54 (W. G. Downs, col.), 16/VIII/54, 8/IX/54 and 13/VI/54 (T. H. G. Aitken, col.). Previously reported by Lane (1953) from Mayo, Pointe-a-Pierre Ward. Known elsewhere from Puerto Rico, Venezuela, Panama, Peru, Argentina and the following Brazilian states: São Paulo (type locality), Minas Gerais and Rio de Janeiro (Lane 1953).

Bionomics.—Adults captured by us at a white light trap between 17:30 and 19:15 hours.

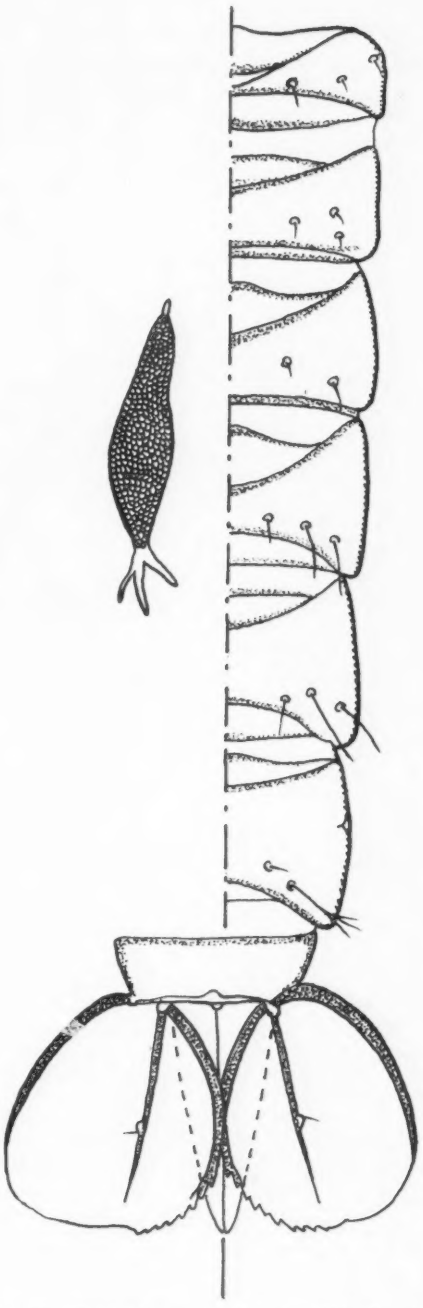

FIG. 17. *Sayomyia souzai* (Lane), 1939—Pupal respiratory tube and abdominal segments II–VIII (E. B. Ferraz, del.).

Sayomyia souzai (Lane) 1939

Chaoborus souzai Lane, 1939, Bol. Biol. (n.s.), 4: 112.
Chaoborus (*Sayomyia*) *souzai* Lane, 1942, Rev. Ent., 13: 147.
Sayomyia souzai (Lane), 1953, Neotrop. Culicidae, 1: 105.

As a large series was obtained a study of variation was possible and is given below.

The mesonotum is quite variable. The mesonotal spots, one in middle anteriorly and two lateroposteriorly, may be so slight that they nearly disappear in some specimens, in others they show as three distinct marks. The mesial spot may be lighter in the middle so that it seems divided. The wings show the same amount of variation and a second mesial faint marking may appear in some specimens, in others there is only a mark on the 5th vein and occasionally such a mark may be so faint as to be nearly indistinct. The abdominal marks are puzzling as they may coalesce and form basal segmental bands; the majority of specimens, however, have a large number of spots. The mid femur has a distinct black apical band which may be brown in some specimens. Male genitalia with basal two thirds of basistyles white, apical third together with dististyles black.

FIG. 18. *Sayomyia souzai* (Lane), 1939—Larval head with pharyngeal basket everted and detached pre-labral appendage (T. H. G. Aitken, del.).

Pupa.—Tube elongate, expanded before middle (index of length to greatest width about 4: 1), reticulate, apical spiracular opening greatly constricted and elongate (Fig. 17). Cephalothorax with brown mottling, setae inconspicuous, integument minutely spiculose, particularly heavy on dorsum between tubes.

Abdomen (Fig. 17) long, uniform, transparent white except for brownish coloration along intersegmental borders—more widespread on segment I; integument minutely spiculose, particularly heavy on mesiodorsal surface of distal segments and extending down onto basal portion of paddle membrane and mid and outer ribs; chaetotaxy largely simple and inconspicuous, but the setae arising from rather prominent plaques. Paddle slightly longer than broad (index 1.25: 1), with three ribs, the mid rib straight and thinner than others and closer to inner rib; the outer and mid ribs subequal in length and shorter than inner rib which is strongly curved and serrate on distal half; a single hair arises beyond middle of mid rib.

FIG. 19. *Sayomyia souzai* (Lane), 1939—Larval left mandible and maxilla (T. H. G. Aitken, del.).

Larva.—General conformity typical of other *Sayomyia* larvae. Head with three dorsal, multiple tufts (asymetrically branched), decreasing in size anteriorly (Fig. 18). Antenna terminating in five curved setae, four slightly longer than antenna and the fifth (most mesial) about two-thirds length of others. Post-antennal filaments five pairs, basally bent and same length as

antenna proper. Prelabral appendages two, length to greatest width about 5: 1, fringed. Mandible (Fig. 19) with three prominent teeth, the first (most anterior) shortest, and the second longest, a supplementary weaker tooth between 2 and 3 and a marginal row of 3 to 5 smaller teeth beyond and dorsal to third; three prominent setae and a spur proximal to first tooth; mandibular fan 8- to 9-rayed. Maxilla with a prominent seta on a cone-like tubercle and a shorter one posteriorly. Labrum setose distally. Caudal fan with 18 feathered rays. Dorsal caudal tufts feathered, two pairs, the more dorsal pair shorter. Anal gills four, short and pointed.

Distribution.—Trinidad (B.W.I.) — Charuma Forest, Tabaquite 20/X/54, 17/XI/54, 26/I/55 (T. H. G. Aitken, col.); St. Patrick Estate, Arima Valley 2/XI/54 (T. H. G. Aitken, col.); Arena Forest, San Rafael 19/VIII/54 (T. H. G. Aitken, col.); Rio Grande Forest, Sangre Grande, 10/X/55, 25/X/55 and 17/I/56 (T. H. G. Aitken, col.); and Tucker Valley, United States Naval Station, 27/X/55. The species has been previously reported from Cunupia, Trinidad (Lane 1953). Elsewhere Lane records it from Panama, and the Brazilian states of Pará (type locality) and Minas Gerais.

Bionomics.—Our larval and pupal material has come from wooden tubs set out in densely shaded forests as well as from shallow forest ground pools. On occasion, adults have been taken in light traps between 17:45 and 19:15 hours as well as overnight.

SUMMARY

Sixteen species of Trinidadian Chaoborinae have been studied, 11 species of *Corethrella*, 2 of *Lutzomiops* and 3 of *Sayomyia*. Of these, 2 Corethrella are new and the allotypes of 6 others are described. In addition the immature stages of 4 *Corethrella*, one *Lutzomiops* and 1 *Sayomyia* are described. The list of known Trinidadian Chaoborinae has been increased by 11 species. Biological notes are included.

THE ABUNDANCE CYCLES OF ARBOREAL MOSQUITOES DURING SIX YEARS AT A SYLVAN YELLOW FEVER LOCALITY IN PANAMA[1]

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INTRODUCTION

The problem of how sylvan yellow fever crossed from eastern Panama through the Canal Zone area and into western Panama and Central America in the period since 1948 has been a subject of much interest. Recent papers which deal with the matter in part have been those of Trapido, Galindo and Carpenter (1955) and Trapido and Galindo (1956a). It has been pointed out that the forest canopy mosquito fauna known to be involved in the transmission of sylvan yellow fever becomes depauperate during the dry season characteristic of the Pacific slope, and that the gap in the mountain system marked by the location of the Panama Canal is an area of transition from the wet and dry deciduous forest conditions of the Pacific slope to the wet rain-forest conditions of the Caribbean slope of western Panama. But on the Pacific side of eastern Panama there is also a gradual transition from tropical deciduous forest in the lowlands and on lower slopes to climatic and vegetation conditions approaching those of tropical rain-forest on the upper slopes (Trapido and Galindo, 1956b). The purpose of the present communication is to present the results of six years of collecting forest canopy mosquitoes at Cerro La Victoria, some 15 miles east of the Panama Canal, and to demonstrate the magnitude of the year to year fluctuations in the populations of these mosquitoes as this may be related to the maintenance of the sylvan yellow fever cycle in the area.

The two stations at La Victoria from which our data are drawn were at elevations of 400 and 1,200 feet, and were thus high enough to be in forest intermediate in appearance between deciduous forest and rain-forest, although the dry season at these localities is sufficiently marked to meet the climatological definition of tropical wet and dry climate associated with deciduous forest. This was an area where sylvan yellow fever occurred late in 1948.

The method of making the collections on which this report is based have been described previously (Galindo, Trapido and Carpenter, 1950). In the initial studies two crews of collectors were used to man the field stations from 8:00 a.m. to 5:30 p.m., but in subsequent years a single crew made collections during the hours when experience had shown the diurnal mosquitoes to be most active, *i.e.*, the hours between 9:00 a.m. and 3:00 p.m.

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So that the population densities from different years might be validly compared, the data from the first year of collecting was reworked to include only the captures made between the hours of 9:00 a.m. and 3:00 p.m. For the present study there are considered only the collections taken attacking human baits stationed on platforms in the forest canopy, because it is in the canopy that the mosquitoes of interest to us are most abundant, and also because it is in the canopy that the sylvan yellow fever transmission cycle is presumed to be carried on among susceptible mammal hosts by arboreal mosquitoes. A full account of the diurnal forest mosquitoes of Panama has already been given (Trapido, Galindo and Carpenter, 1955), and the present paper will deal only with those mosquitoes known or suspected as vectors of sylvan yellow fever. These are five in number as follows: *Haemagogus equinus* Theobald, *Haemagogus lucifer* (H., D. and K.), *Haemagogus spegazzinii falco* Kumm et al., *Aedes leucocelaenus clarki* G., C. and T., and *Sabethes chloropterus* (Humboldt). The nature of the evidence implicating these species in yellow fever transmission has been reviewed recently by Trapido and Galindo (1956a). There is no direct evidence with regard to *Haemagogus lucifer*, but this species is included since it appears increasingly likely that all *Haemagogus* are capable of transmitting the disease under suitable conditions (Galindo, Rodaniche and Trapido, 1956).

As the annual abundance cycles of arboreal forest mosquitoes are dependent on rainfall we will first present these data.

RAINFALL

The available rainfall data are those published by the Section of Meteorology and Hydrography of the Panama Canal Company. We did not, unfortunately, operate rain gauges at the sites of our forest mosquito collecting stations, but it is quite certain that the rainfall there was intermediate in amount between that reported for El Jefe and Balboa Heights. The annual rainfall at El Jefe is more than twice that at Balboa Heights since the former station is much higher than the latter, but the monthly distribution over the year at the two stations is very much the same. The average rainfall for these two stations during the six years of the study, together with the minima and maxima are shown in Table I. The "dry season" is the low sun period from January through April. At El Jefe only 7.3 percent and at Balboa Heights only 6.3 percent of the annual precipitation fell during these four months. From the point of view of our interest in the maintenance of yellow fever transmission we are particularly interested in the rainfall variation during the dry season, the period when arboreal mosquito populations are minimal. Inspection of the data in Table I shows that the magnitude of the fluctuations from year to year is very great. If we take for an example the month of February, we see that at El Jefe during the six year period of the study the rainfall varied from 0.61 inches to 13.10 inches, and at Balboa Heights from only a trace to 2.49 inches. It may be readily appreciated from these data how variable are the conditions from year to year for the production of the tree hole breeding arboreal mosquito populations.

TABLE I

SUMMARY OF VARIATION IN RAINFALL DURING THE SIX YEARS 1949 THROUGH 1954

STATION		JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	YEAR
[1]El Jefe, Panama.....	Max.	9.79	13.10	2.58	6.64	31.69	20.78	21.14	21.47	26.54	24.72	27.51	16.77	169.21
	Av.	2.91	3.25	1.02	3.84	22.51	17.85	15.44	17.59	18.22	21.36	16.59	10.80	151.38
	Min.	0.66	0.61	0.35	1.29	15.61	14.71	13.48	13.75	15.20	16.61	6.08	6.77	132.23
[2]Balboa Heights, Canal Zone.....	Max.	2.66	2.49	1.89	4.89	15.06	13.26	12.14	8.49	9.79	14.81	13.62	13.46	84.03
	Av.	0.92	0.87	0.72	2.12	9.85	8.47	7.64	6.60	7.33	12.49	10.04	6.35	73.39
	Min.	0.15	T	0.11	0.07	8.22	3.97	4.97	2.90	3.79	7.08	6.28	3.80	63.86

[1]Elevation 3,200 feet. [2]Elevation 100 feet.

LONG TERM VARIATIONS IN FOREST CANOPY MOSQUITO POPULATIONS

The results of the forest canopy mosquito collections at Cerro La Victoria are summarized in Table II and in figure 1. These data represent the average capture rates per ten man hours at two stations, with the exception of 1951 when only one of the stations was operated. The collections are unfortunately incomplete for the first five months of certain of the years. In Table II months during which no collecting was done are indicated by dashes. It will be seen in this table that for January there are three years of data, for February, March and April four years, and for May five years.

On reference to figure 1 it can be seen at once that for each species there appears a characteristic average annual abundance cycle. The configuration of these abundance cycles in general follows that reported for the first year of study at these stations (Galindo, Trapido and Carpenter, 1950). The populations of *Haemagogus equinus* are the most rapid in building up after the beginning of the rainy season in May. The peak densities of

Haemagogus lucifer, *H. spegazzinii falco*, and *Sabethes chloropterus* appear somewhat later, in June and July. The apparent low densities of *Aedes leucocelaenus clarki* are somewhat deceptive, since at La Victoria this species is not as arboreal as the others and the data here include only the individuals taken in the canopy.

The principal interest in the data presented concerns the year to year variations in the densities of the monthly captures. Numerical values are shown in Table II, but the magnitude of the variations may best be appreciated by reference to figure 1 in which the monthly maxima and minima (as well as the means) are graphically portrayed. It may readily be appreciated from a review of these data that yellow fever transmission potential must vary greatly from year to year, although it would also appear that if all these species are capable of transmitting the disease there are probably high enough densities of one or the other species during the rainy season months to maintain the cycle. It is evident that the dry season is the critical time of year when the maintenance of the yellow fever transmission cycle must be precarious. During the four dry season months (January through April) *Haemagogus spegazzinii falco* and *Aedes leucocelaenus clarki* were completely lacking. *Haemagogus equinus* and *H. lucifer* were present, although at very low densities, during each of these months in one year or another, but not during all four months in any one year. The only species which appeared during every month in any one year was *Sabethes chloropterus*.

TABLE II

SIX YEAR ABUNDANCE CYCLES OF FIVE MOSQUITOES KNOWN OR SUSPECTED AS SYLVAN YELLOW FEVER VECTORS
CERRO LA VICTORIA, PANAMA
(SHOWN AS NUMBER OF EACH SPECIES PER TEN MAN HOURS OF COLLECTING IN THE FOREST CANOPY.)

SPECIES	YEAR	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.
Haemagogus equinus	1949	—	0.0	0.0	2.0	13.1	41.8	18.3	3.6	8.2	1.8	4.4	0.9
	1950	0.4	0.0	0.0	0.4	14.8	20.5	9.2	4.6	1.7	4.6	4.0	1.8
	1951	—	—	—	—	—	9.5	0.8	0.0	1.9	0.8	1.9	0.0
	1952	—	—	—	—	73.2	42.1	22.2	5.7	8.6	8.6	6.4	9.7
	1953	3.0	4.9	0.0	1.1	38.0	16.3	14.7	3.4	12.2	16.1	6.6	1.5
	1954	0.0	0.0	0.8	0.0	56.9	29.5	13.3	13.1	1.4	1.9	2.8	1.4
	Av.	1.1	1.2	0.2	0.9	39.2	26.6	13.1	5.1	5.7	5.6	4.4	2.6
Haemagogus lucifer	1949	—	0.0	0.0	0.0	10.8	20.9	35.7	13.0	19.7	17.1	25.4	7.2
	1950	0.8	0.0	1.3	0.2	4.6	8.3	12.8	8.3	3.8	3.5	3.6	2.9
	1951	—	—	—	—	—	2.7	3.3	2.6	1.9	0.8	5.4	0.5
	1952	—	—	—	—	17.7	25.3	24.1	6.6	7.7	6.7	6.6	6.4
	1953	1.8	0.8	0.0	0.3	9.7	10.1	16.3	10.1	17.3	12.1	15.6	13.1
	1954	1.7	0.0	0.0	0.0	17.8	14.4	26.3	18.8	4.0	4.4	7.8	3.4
	Av.	1.4	0.2	0.3	0.1	12.1	13.6	19.8	9.9	9.1	7.4	10.7	5.6
Haemagogus spegazzinii falco	1949	—	0.0	0.0	0.0	0.0	14.5	16.1	2.6	8.3	4.7	10.2	3.9
	1950	0.0	0.0	0.0	0.0	0.7	3.3	5.9	1.8	1.3	0.5	1.1	0.6
	1951	—	—	—	—	—	1.4	1.7	0.9	1.4	0.0	0.8	0.0
	1952	—	—	—	—	3.8	6.8	2.1	0.7	0.4	0.9	0.9	0.3
	1953	0.0	0.0	0.0	0.0	0.9	1.0	3.3	1.0	1.3	4.3	2.2	1.8
	1954	0.0	0.0	0.0	0.0	3.1	11.7	2.4	3.8	0.6	0.8	1.0	0.4
	Av.	0.0	0.0	0.0	0.0	1.7	6.5	5.3	1.8	2.2	1.9	2.7	1.2
Aedes leucocelaenus clarki	1949	—	0.0	0.0	0.0	2.2	2.4	3.9	2.2	2.2	2.2	1.1	1.7
	1950	0.0	0.0	0.0	0.0	0.0	0.3	0.6	1.6	1.3	0.4	1.2	0.0
	1951	—	—	—	—	—	0.5	0.4	0.4	5.2	1.5	7.7	1.1
	1952	—	—	—	—	1.0	3.5	4.6	1.1	0.9	0.7	0.8	0.3
	1953	0.0	0.0	0.0	0.0	0.0	0.3	1.9	2.6	2.1	6.9	4.1	3.1
	1954	0.0	0.0	0.0	0.0	0.0	2.8	5.3	4.8	1.3	1.0	1.0	0.6
	Av.	0.0	0.0	0.0	0.0	0.6	1.6	2.8	2.1	2.2	2.1	2.7	1.1
Sabethes chloropterus	1949	—	0.0	0.4	1.4	6.3	30.9	32.2	32.0	12.2	8.6	11.8	3.8
	1950	1.8	0.0	1.5	1.5	3.2	7.7	16.4	10.4	4.5	2.0	1.2	1.8
	1951	—	—	—	—	—	5.0	1.3	1.3	2.4	0.4	2.3	1.1
	1952	—	—	—	—	5.2	17.9	51.1	29.9	19.0	9.9	3.6	6.8
	1953	2.0	0.5	0.5	0.3	8.6	9.1	22.8	16.3	10.0	9.7	4.1	1.8
	1954	0.2	0.0	0.0	0.2	5.4	19.5	29.1	19.1	4.1	1.2	3.4	1.2
	Av.	1.3	0.1	0.6	0.9	5.7	15.0	25.5	18.2	8.7	5.3	4.4	2.8

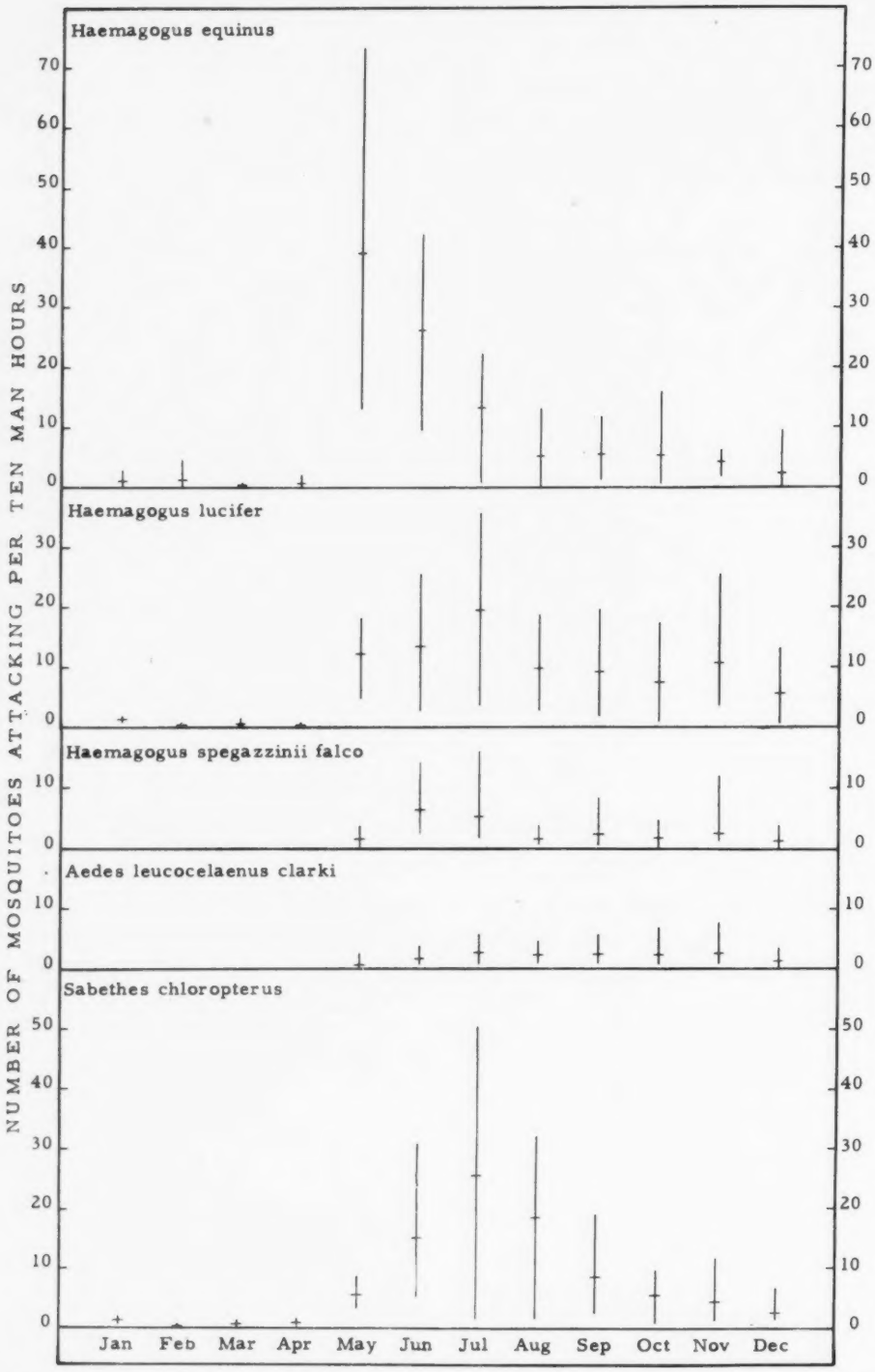
Haemagogus equinus
Haemagogus lucifer
Haemagogus spegazzinii falco
Aedes leucocelaenus clarki
Sabethes chloropterus
NUMBER OF MOSQUITOES ATTACKING PER TEN MAN HOURS
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

FIGURE 1. Annual abundance cycles of five arboreal tree hole breeding mosquitoes known or suspected as sylvan yellow fever vectors, based on six years of collecting. Shown are the maximum, minimum and mean numbers taken per ten man hours, by months.

The three species of *Haemagogus* and *Aedes leucocelaenus clarki* are aedine mosquitoes with eggs which resist desiccation, and their survival during the dry season in this state poses no special problem. But as the present understanding is that there is no transovarian passage of yellow fever virus (Whitman, 1951) the means by which the virus is maintained through dry season is a very live issue.

Sabethes chloropterus also utilizes rot holes in trees for breeding, but the eggs do not resist desiccation. For survival through the dry season this species must either find breeding places or else be so long lived as to persist as adults from one rainy season to the next. We have previously shown that this species does possess a mechanism for breeding through the dry season (Galindo, Carpenter and Trapido, 1951). That is, the species lays its eggs preferentially in tree holes which have a small aperture opening into rot holes with a large water holding capacity. Such water containers are protected from the desiccating winds of dry season and are able to retain water through this unfavorable time of year. This finding has been confirmed in the laboratory by two of the authors (P. G. and H. T.) who have succeeded in colonizing the species. Even under caged conditions it has been found that *chloropterus* will only lay eggs in numbers in water containers which are closed at top and have a small aperture on the side (*i.e.*, covered bamboo sections with a hole one inch in diameter in the side). The colony experience has also been that *Sabethes chloropterus* is longer lived than the species of *Haemagogus* which have been studied in the laboratory. Thus it appears that both means of surviving the dry season mentioned above, are utilized.

While it is true that the five mosquitoes dealt with here are predominantly arboreal, the forest at Cerro La Victoria has a more open aspect than is seen in true tropical rain-forest, and the vertical stratification of these species is not so marked as in forest of the latter type. The dry season is the period of maximum leaf loss at Cerro La Victoria. At this time of year the difference between the microclimate in the canopy and at ground level is least pronounced, and there is some tendency for the arboreal species to appear less well stratified in the forest. This phenomenon had been noted by Bates (1944) for *Haemagogus* in Colombia. At Cerro La Victoria we found this to be particularly true of *Sabethes chloropterus* in some years. It was most clearly shown in 1949 at a time when collections were made at platforms midway up the trees as well as on the ground and in the canopy. The decline in the catch in the canopy and the increase in the catch at ground level and at the middle platform during dry season may be seen in the following tabulation in which the proportions of the *chloropterus* in catch at the three levels are shown as percentages.

	Ground	Middle Platform	Canopy Platform
Dry Season.....	23%	61%	16%
Rainy Season.....	8%	46%	46%

DISCUSSION AND SUMMARY

The data here summarized demonstrate that in an area of Panama through which sylvan yellow fever passed, where the vegetation cover is transitional between tropical deciduous forest and tropical rain-forest, there are substantial year to year fluctuations in rainfall and correspondingly great fluctuations in the densities of the arboreal mosquitoes known or suspected to be yellow fever vectors. During the dry season characteristics of this forest type the arboreal mosquito fauna becomes rare. But it is shown that there is enough variation in mosquito populations so that during the four month dry season at least some of the vector species are present during each month in some years. The tendency toward a lesser vertical stratification of the arboreal mosquito fauna in dry season means that these mosquitoes are actually not quite so scarce as the tabulations of the canopy catch summarized might indicate. Of the mosquitoes known or suspected as vectors of sylvan yellow fever, *Sabethes chloropterus* was the one species taken in the canopy during all months in any one year.

ACKNOWLEDGEMENTS

The long term forest mosquito collections at Cerro La Victoria were made as a cooperative enterprise of the Gorgas Memorial Laboratory and the 25th Preventive Medicine Detachment of the U. S. Army Caribbean. The workup of the data was in part accomplished while one of the authors (H. T.) was on a short tour of active duty with the U. S. Army Caribbean.

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THE DORSAL GLANDS AND VENTRAL GLANDS OF PERIPLANETA AMERICANA (L.) (BLATTIDAE, ORTHOPTERA)[1]

CHENG LIANG

INTRODUCTION

For a long time it has been known that cockroaches leave their excreta over their runways, spoiling articles of food which retain their unpleasant roachy odor even when cooked. According to Marlatt (1915), this odor comes partly from their excrement, but chiefly from the dark-colored fluid exuded from their mouth. It also, in part, comes doubtlessly from the segments of the abdomen, which secrete an oily liquid possessing a very characteristic and disagreeable odor. It has long been known that cockroaches possess a pair of dorsal glands between the terga and a pair of ventral glands between the sterna of the abdomen. There is no doubt that these glands are secretory in nature. Nothing is known about the composition of the secretions.

The dorsal and ventral glands and other glandular structures of *Blatta orientalis* L., *Periplaneta americana* (L.) and *Blattella germanica* (L.) were dissected and extracted with various organic solvents with the intention of determining their function in an olfactometer. During dissection, a white, cylindrical organ was found which Miall and Denny (1886) described as the conglobate gland. This structure which lies under the sixth abdominal ganglion just below the ventral gland is about 4 mm. in length in the male of both *B. orientalis* and *P. americana*. The function of this gland will receive future attention.

Sections were made of the dorsal and ventral glands in both male and female *P. americana* and *B. orientalis*. The findings in *P. americana* are related herein.

PREVIOUS WORK ON THE GLANDS OF COCKROACHES

Glandular structures were first described from the dorsal abdominal region of cockroaches by Gerstaecker (1861) who found two pairs of small dorsally located eversible glandular vesicles in a species of *Corydia* between the first two abdominal segments. These structures were present in adult males and females, but not in nymphs. The function of these glands was unknown. Unfortunately the material, obtained by Gerstaecker from other sources, did not receive accurate histological investigation.

A pair of dorsal glands between the fifth and sixth terga, present in both sexes of the adult and in all stages of the nymph of *Blatta orientalis*, was first described by Minchin (1888), whose observations were confirmed by Hasse (1889). Their histology has been further studied by Oettinger (1906) and by Konček (1924). Secretory cells underlie the surface dermal layer of these glands.

In 1890, Minchin also mentioned that he found dorsal glands in males of *Periplaneta americana* and *P. decorata* Brunner (one male nymph). In *P. americana* these glands were found to be constricted almost as described in *B. orientalis*, but in *P. decorata* they were a little "shallower" and of greater lateral extent, and lined with branched hairs. Minchin found in this species an additional gland, opening by a tubular duct under the intersegmental membrane between the fifth and sixth terga above the glandular pouch on each side, and extending forward into the body cavity. The gland and its duct were proliferations of the hypodermis, and there was no invagination of the cuticle.

Kraus (1890) further described similar glandular organs of a small *Aphlebia bivittata* Brullé. These were two glandular organs, lobed in the shape of horse shoes and occuring only in the male in a groove in the back of the seventh abdominal segment. They were similar to previously described dorsal glands, having a tuft of dense but unbranched hairs. Wattenwyl (1882) was first to point out round or long openings surrounded by short coarse hairs on the sixth and seventh dorsal segments in the male of *Ectobia* and *Blattela* (Phyllodromia).

A pouch-like structure was first described by Haase (1889) in *B. germanica*. Minchin (1890) observed such glands, and Oettinger (1906) sup-

EXPLANATION OF FIGURES CONCERNING DORSAL AND VENTRAL GLANDS OF *P. Americana*.

FIG. 1. Ental aspect of the dorsal glands of a female. 10X.

FIG. 2. Parasagittal section through dorsal gland and its external opening of female. 80X.

FIG. 3. Enlarged view of glandular cells shown in fig. 2. 311X.

FIG. 4. Detail of secretory cells of dorsal gland showing tubula connecting with hair shown in fig. 5. 1967X.

FIG. 5. Parasagittal section through dorsal gland without cuticle of male. 377X.

FIG. 6. Ental aspect of the ventral glands of a female. 10X.

FIG. 7. Parasagittal section through ventral gland and its external opening of female. 168X.

Fig. 8. Parasagittal section through ventral gland and its external opening of male. 123X.

FIG. 9. Enlarged view of glandular cells shown in fig. 8. 287X.

KEY TO ABBREVIATIONS

B.M., basement membrane; C.G., conglobate gland; D.G., Dorsal Gland; E.O., external opening; G.C., glandular cell, H., hair; L., Lumen; Li., intimal lining; N., nucleus; S., sternum; T., tergum; Tu., tube; V., ventral gland.

[1]Accepted for publication November 16, 1955.

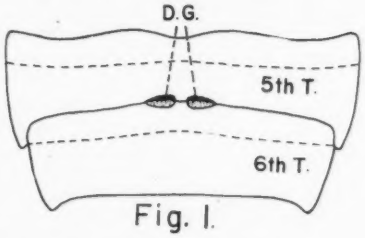


Fig. 1.

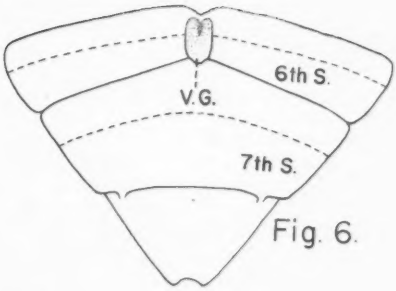
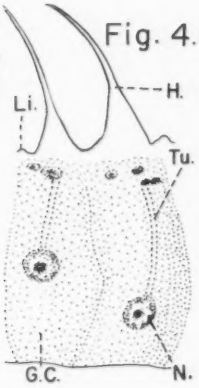


Fig. 6.

Fig. 2.



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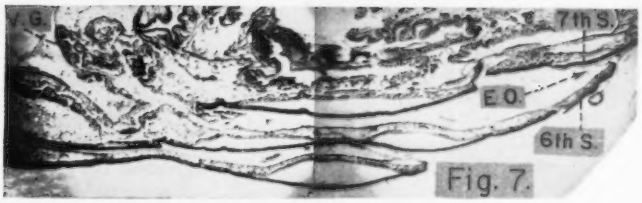

Fig. 7.

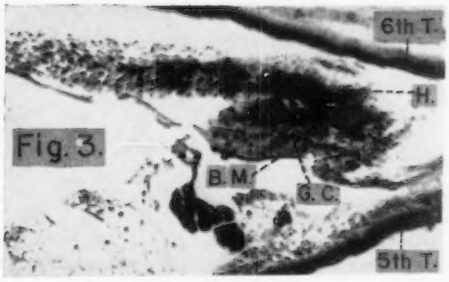

Fig. 3.

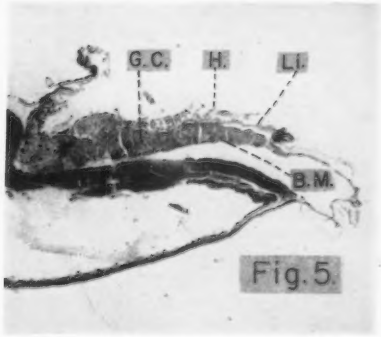

Fig. 5.

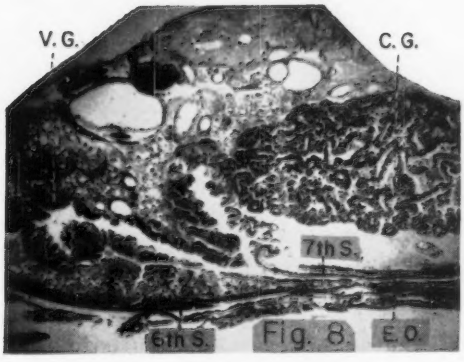

Fig. 8.

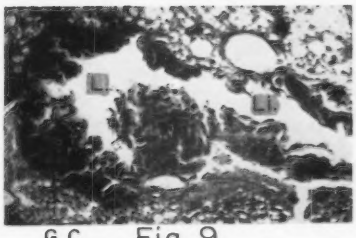

Fig. 9.

plemented the findings of Haase and Minchin in many respects of the basis of detailed investigation. These glands were found only in the adult male and were more complicated in structure than previously named forms. They were marked by two deep irregular depressions of the intersegmental membranes at the bases of the seventh and eighth tergal plates, into each of which depressions opened a pair of glands, the first between segment VI and VII, the second between segment VII and VIII. The glands of *Blattella* were similar to those of *Blatta* (*Periplaneta*) though located on different segments.

Only limited observations have been made on the ventral glands of cockroaches. Harrison (1906) described a pair of such glands in *B. orientalis* located between the sixth and seventh sterna in the region of the last abdominal ganglion. He called these "sternal glands", but in light of the prevalence of the term "dorsal glands", it seems that "ventral" is a better suited term.

MICROTECHNIQUE

Size and transparency of the glands, and problems inherent in attempting to section insect cuticle, make the adoption of certain techniques advisable. To distinguish the glands easily in gross dissection, the whole cockroach or a piece of tissue may be fixed in ordinary fixative for 24 hours and subsequently washed. Excellent sections may be made of the glands alone but these do not elucidate their connection with the integument and other tissues. Mechanical difficulties encountered in sectioning integument were most readily overcome by using newly molted roaches. Paraffin embedded portions of gland and related integument can be soaked in a 1 percent solution of detergent such as Dreft for 24 hours (after Pearlman and Cole, 1951) to improve sectioning; however material from freshly molted roaches proved better.

Conventional fixation, embedding, and staining techniques are adequate for general observations. Bouin's or FAA are generally satisfactory although Helly's appeared to be the best fixative for cytoplasm. Paraffin of 50° C. melting point was used; a higher melting point material (such as 56–58° C.) would probably be more desirable. For ordinary purposes iron-hematoxylin stain with an eosin counterstain is adequate. Sudan black was used for fat detection, and Muci carmine to determine if mucous cells were present.

STRUCTURE AND HISTOLOGY OF THE DORSAL AND VENTRAL GLANDS

1. THE DORSAL GLANDS

A. Female.—The dorsal glands of the female of *P. americana* have not been described before. Between the fifth and sixth abdominal segments of the female of *P. americana*, there are two pouch-like invaginations of the cuticle, one on each side of the middle line. These are the dorsal glands (fig. 1) described in several other species of cockroaches. If the fifth abdominal tergite is taken off, one may see two pouch-like organs lying under the intersegmental membrane. The opening of each gland is nearly as large as its width. During dissection the gland is hard to recognize, because the pouches are somewhat transparent like the intersegmental membrane. If the entire stained area is inspected under the binocular or compound microscope many hairs may be seen overlying the glandular cells. When the back of a cockroach is pressed, a sticky fluid which lacks unpleasant odor is exuded on the back of its sixth tergite. It is relatively easy to dissect out the complete organ, since there is no muscle attached to the gland pouch.

Cross-sections (fig. 2) show that the gland is folded between the fifth and sixth abdominal terga. There is a basal membrane continuous with that of the hypodermis of the terga, which delineates the pouch from the body cavity. Columnar glandular cells are found next to the basement membrane, being two or three layers deep in the middle where the gland is thickest. After fixation with Bouin's the nuclei of these cells are large and oval or pointed and irregular, each possessing a distinct nucleolus. The cytoplasm shows a very fine granular consistency. Some of these columnar cells possess a tube extending from nucleus to the boundary of the cell. A layer of small flattened cells with irregularly-shaped nuclei lies next to the pouch lining and is undoubtedly a continuation of the tergal hypodermal layer. The lining of the pouch is continuous with the cuticle of the terga and has many hollow and weakly sclerotized hairs attached to it. These hollow hairs are swollen at the base and may serve as tubes through which glandular fluid is exuded.

B. Male.—The dorsal glands in the male of *P. americana* (fig. 5) lie in the same position as in the female. They are almost the same or a little smaller than those in the female. The hollow hairs are also swollen at the base, but the nuclei of the glandular cells are round or oval in shape (fig. 4) instead of irregular as in the female.

2. THE VENTRAL GLANDS

The ventral glands have not been described before in either sex of *P. americana;* it is a newly discovered organ in this animal.

A. Female.—The ventral glands are composed of a pair of small oval pouches which are present between the sixth and seventh sterna below the last abdominal ganglion (fig. 6). Two tracheal trunks and many tracheoles may be seen to cover these organs when the nerve cord is removed. They appear as two lobes projecting anteriad from a median structure. At their base the two lobes unite and are attached to the crescent-shaped anterior margin of the seventh sternite with an exterior passage consisting of a single aperture on the ventral surface of the cockroach between the sixth and seventh sterna.

In *Blatta orientalis* this has been described as two separate structures in the nymph, which unite and form a single bilobed structure in the adult (Qadri, 1938). The crescent-shaped anterior edge of the seventh sternite is more heavily sclerotized than the adjacent integument.

In parasagittal section (fig. 7) each gland shows several folds. The anterior portions of each gland possess a layer of columnar cells with large rounded or hexagonal nuclei. These cells are secretory and contain a frail tube beginning near the nucleus and continuing toward the lumen of the gland. Following fixation with Bouin's their cytoplasm is largely granular and at times contains a small or very large vacuole, especially towards the lumen of the gland. Between the glandular cells and the lumen of the gland lies an irregular cell layer, in which the nuclei are irregular in shape and lie next to the lumen of the gland. The nuclei nearly fill these cells which are apparently continuous with the hypodermis of the sterna, with a much folded intimal lining next to them. This is the lining of the gland which has fine hairs attached to it.

B. Male. The ventral glands of male *P. americana* (fig. 8) are the same as the female's except for the fact that the vacuoles in the cytoplasm of glandular cells are never as large.

DISCUSSION

From the fact that if one of the pouches of the dorsal glands be macerated for a sufficient time in 30 percent caustic potash solution, nothing would be left but the hairs and the lining on which they stand, it is evident that these hairs and the lining of the gland contain chitin. The hairs seem to develop as simple foldings of the cuticle. This lining, the glandular cells, and the basal membrane, obviously are a continuation of the tergal integument. The glandular cells appear to be derived from proliferated hypodermal cells. Therefore we may say that the dorsal gland is an invagination of the intertergal membrane in which underlying cells have become glandular in nature.

The lining of the ventral gland also contains chitin. The lining and the hypodermis with its basal membrane are also continuations of the sterna. Therefore the ventral gland may be considered as an enlarged part of the intersegmental membrane in which hypodermal cells are modified to form the two lobed pouches which are the ventral glands. The hairs which are attached to the lining are fine and show some differences from those in the dorsal gland. The intracellular tubes in the secretory cells of the ventral glands resemble those in the dorsal glands.

Since the dorsal and ventral glands are negative to Sudan black, they have no fat cells, unlike conglobate and salivary glands which are positive, and consequently have fat cells. In Muci carmine, no glands stained red, an indication that they have no mucous cells.

Nothing is known about the function of the dorsal and ventral glands of both sexes in *P. americana*. The fluid produced by them does not have an odor detectable to humans. Additional work on the development of these glands may furnish clues to their function.

SUMMARY

1. Two pouches located between the fifth and sixth abdominal terga of both sexes of *P. americana*, and the bilobed pouch between the sixth and seventh abdominal sterna of this same insect, contain glandular cells and are known as the dorsal glands and the ventral glands respectively.

2. Both structures appear to be invaginations of the intersegmental membranes in which the hypodermis has secreted a cuticular lining with hairs on its surface. Glandular cells with internal ducts are associated with hypodermal layer.

3. The dorsal and ventral glands are similar in location and structure in both sexes of *P. americana*.

4. The nature of the secretions and their function remains a matter of conjecture.

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THE EUROPEAN CORN BORER, PYRAUSTA NUBILALIS (HUBN.), AND ITS PRINCIPAL HOST PLANT.

I. ORIENTATION AND FEEDING BEHAVIOR OF THE LARVA ON THE CORN PLANT[1]

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The European corn borer is a non-host-specific insect, and has been reported feeding on over 200 different plant species (Caffrey and Worthley 1927, Hodgson 1928, and others). The borer's native Eurasian host was probably wild hop or one of the larger grasses, such as millet (Caffrey and Worthley 1927). Corn, being of American origin, could not have been the native host, but has proved to be the preferred host wherever the geographical distributions of the plant and insect coincide. The broad range of possible host plants is of considerable significance to the study of the relationships between the borer and the corn plant. It indicates that the plant characteristics required for successful borer establishment and growth are present in a large number of plant species and are not peculiar to corn alone.

Host selection, from the standpoint of the selection of the individual plant to be attacked, is not ordinarily a larval function. Such selection is made by the ovipositing European corn borer moth. Borer moths will lay their eggs on a large number of different plant species. Caffrey and Worthley (1927) list rhubarb, beans, spinach, potatoes, hemp and many other plants as oviposition hosts. It is quite apparent, however, that corn is the preferred host, and that oviposition on other plant species is negligible when suitable corn is present (Thompson and Parker 1927, Caffrey and Worthley 1927, Clark 1939, Neiswander 1945). Moore (1928) detected, but did not identify, a steam-volatile oil in corn leaves which was attractive to borer moths.

The eggs of the European corn borer are laid on the underside of the leaves of the host plant, and the larvae which issue from the eggs tend to survive or die on the plant on which they hatch. On corn, there may be some plant-to-plant migration, but such movement is apparently negligible during the feeding stages. Newly-hatched larvae may be dispersed by wind to adjacent plants (Neiswander & Huber 1931), and mature larvae may leave the host plants in significant numbers (Barber 1924, Neiswander and Huber 1931). Although the borer larvae do not select the host plant, they are responsible for the selection of the part of the plant on which to feed. Such selection of feeding sites may be comparable to the host selection displayed by the feeding stages of some more polyphagous insect species.

A newly-hatched borer larva living on a young corn plant is not on a chemically or morphologically stable substrate. The plant's chemical composition varies between hours of daylight and darkness, and also according to its developmental stage. The plant's physical form changes strikingly as it reaches successive developmental stages of tasselling, silking, and kernel formation. Nor can the insect be considered a stable entity; like the plant, it is undergoing developmental changes. The selection of a feeding site is undoubtedly the resultant of a number of factors, some of which are the chemical and physical characteristics of the plant, and the rest of which are the behavior responses of the insect to its plant environment. As the characteristics of the corn plant change, the feeding sites of the infesting borers also change. The observations and results of many workers (Caffrey and and Worthley 1927, Neiswander *et al.* 1928a and b, Beard 1941 and 1943, Beard and Turner 1942, Patch 1943, Batchelder 1949, Chiang and Hodson 1953, and Cox 1955) show that the feeding habits of the borer follow a consistent pattern, the nature of which is dependent on the plant growth stage at the time of initial infestation. First instar larvae will make their initial attack on leaf tissue within the whorl, enclosed or partially enclosed tassel, ear shoots, ligules and leaf sheaths, and ear husks and silks, depending on the availability of these structures and the location of the eggs from which the borers hatched. As the plant grows and its structure changes, the sites of borer feeding shift: 1) as the tassel elongates, borers feeding within the whorl attack the enclosed tassel; 2) as the tassel unfurls, the borers feeding on it move downward on the plant to become established in ear shoots, leaf sheaths, and in the tassel stem; 3) as the tassel sheds its pollen and dries out, the borers feeding there move lower on the plant and attack ears, leaf sheaths, stalk, and ear shanks. Larvae hatching on a plant that has tasseled and silked tend to attack the ear via either husks or

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silks, or they feed at the leaf base, in the midrib, or around the ligule and sheath, later entering the stem and ear shank.

As part of a long-term study of the host-plant relationships of the European corn borer, the object of the present research was the identification of the major factors influencing the initial orientation and subsequent feeding habits of the borer larva on the corn plant. As a result of a consideration of the published results of other workers and the author's observations on the behavior of borer larvae under experimental conditions, the following hypothesis was critically tested: The orientation and feeding habits of the European corn borer on the corn plant are determined by the larval behavior characteristics of (1) negative phototaxis, (2) positive thigmotaxis, and (3) positive saccharotrophism, and the relation of these behavior traits to the chemical and physical characteristics of the corn plant. Saccharotrophism is defined here as a tendency to feed selectively on nutritional substrates high in sugar concentration.

METHODS AND MATERIALS

The European corn borer larvae used in this study were newly-hatched and unfed. They were obtained from eggs laid in a laboratory oviposition cage by moths produced from field collected larvae. The corn plants employed were field corn inbreds of the line W204 grown on the University of Wisconsin Farmlands. The plant samples were collected at the same time each day (9 a.m.) and were quick frozen within 30 minutes after collection. The plant tissues were stored in a frozen state until utilized.

The methods employed in determining the feeding reactions of newly-hatched borer larvae have been described in detail in a previous publication (Beck, 1956). The borer larvae were given a choice of agar-based purified diets on which to feed. The dietary media contained water, agar, cellulose, casein, corn oil, sugar and (in some experiments) homogenized plant tissue. In experiments designed to measure the influence of plant tissues on larval feeding, the diets contained an optimum level of sugar, in order to prevent the larvae from reacting to the sugar content of the tissues. The dietary media were cut into round discs and placed at equal distances apart in a circle in a Petri dish. In two choice experiments there were four dietary discs, or feeding stations, per Petri dish, each experimental diet being represented twice. In four choice experiments, eight feeding stations were present, each diet being represented twice, with the relative positions of the different diets randomized according to a table of random numbers. From 30 to 40 borer eggs, which were within a few hours of hatching, were placed on a small piece of blotting paper in the center of the dish. Eight replicate dishes were set up in each experiment. The dishes were in the dark at 30° C. for 24 hours. At the end of that time, counts were made of the number of larvae established at each feeding station. Statistical analysis of the data was by analysis of variance following square root transformation, as recommended by Snedecor (1946) for treatment of small numbers. In the interest of clarity of presentation, the data presented in this paper are expressed as per cent of larvae established on the diets at the end of 24 hours.

Plant tissue samples which were subjected to quantitative analysis of sugars were extracted with 80 percent ethanol for four hours in a micro-soxhlet extractor. The alcohol extracts were concentrated, clarified, and decolorized by the method of Hassid (1936, 1937). The extracts were then subjected to Dreywood's anthrone method for the estimation of total sugars, as described by Morris (1948). The results are expressed as milligrams of sugar (glucose equivalents) per gram of fresh plant tissue. Preliminary experiments on the analytical method showed that neither cellulose nor starch interferred with the analysis of sugar. Recovery experiments showed that known amounts of glucose added to the plant sample were recovered to the extent of from 98 to 105 percent.

RESULTS

THIGMOTAXIS AND PHOTOTAXIS

Previous workers have reported that borer larvae are strongly thigmotactic and negatively phototactic (Neiswander *et al.* 1928a, Bottger 1940). However, both the writer and Chin (1951) have observed that newly hatched first instar borers show a positive phototaxis toward a weak or artificial light. In order to test the reactions of newly hatched larvae to light and contact, and to determine the importance of these reactions to the initial distribution of the larvae on the host plant, experiments were run in which artificial corn plants were employed. The artificial corn plants were constructed with cotton wicking stems, waxpaper leaves and leaf sheaths, and copper wire midribs. The "plant" was made to resemble a young corn plant having two leaves with differentiated ligules and leaf sheaths, two partially expanded whorl leaves, and four unexpanded whorl leaves. The artificial corn plants were infested with borers by fastening egg masses to the underside of lower leaves next to the midribs. The egg masses used were within a few minutes of hatching, and an approximately equal number of eggs were affixed to each of the two lower leaves of each artificial plant. The "plants" were then placed in a dark incubator, under artificial lights, or outdoors in direct sunlight. The time of hatching was noted, and three hours later the plant model was dismantled and the locations of the borers deter-

mined. The results of these experiments are presented as Table I.

These data clearly show that the newly hatched larvae were negatively phototactic and positively thigmotactic. Direct sunlight resulted in all recovered larvae being in the "whorl" or under the "leaf sheaths", indicating a negative phototaxis. Under conditions of total darkness, there was less tendency for the larvae to wander. Of the nearly three quarters of the larvae recovered, two-thirds were still on the leaves on which they hatched. One third were concentrated under leaf sheaths or in the rolled unexpanded whorl leaves, indicating a thigmotactic response. Under artificial light, an intermediate distribution was observed. The results are consistent with the interpretation of Neiswander *et al.* (1928a) that sively in these sites. The results obtained tend to confirm the observations of earlier workers that first instar borer larvae display thigmotaxis and negative phototaxis, at least to strong sunlight, and indicate that these two behavior characteristics are important to the initial orientation of the larvae on the plant.

The parts of the corn plant to be invaded by newly-hatched larvae will depend on the plant's stage of morphological differentiation and on the location of the borer eggs. The larvae tend to become located in the "sheltered" parts of the plant, and whether such shelter is found at leaf base, whorl, developing tassel, leaf sheath, ear shoot, or ear depends on the stage of growth of the host. That larvae of instars later than the first also display thigmotaxis and negative photo-

TABLE I

POSITION OF EUROPEAN CORN BORER LARVAE ON ARTIFICIAL CORN PLANTS THREE HOURS AFTER HATCHING

Environment	Infestation			Percentage Distribution of Recovered Larvae					
				External Plant Parts			Internal Plant Parts		
	Original larvae	Recovered		Leaf of hatch	Other leaves	Total	Under Leaf sheaths	Base of whorl	Total
	(No.)	(No.)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Dark incubator (no light)	45	33	73.4	66.7	0	66.7	27.2	6.1	33.3
Laboratory (artificial light, light breeze)	44	21	47.7	23.8	14.3	38.1	33.3	28.6	61.9
Outside (direct sunlight, light breeze)	40	25	62.5	0	0	0	48.0	52.0	100.0

thigmotaxis and negative phototaxis operate together, the borers' negative reaction to strong light tending to bring them into situations satisfying their thigmotactic instinct.

The behavior and distribution of the borer larvae on the artificial corn plants was quite similar to that of borers hatching on small corn plants under field conditions. Huber (1936) found that three hours after hatching on small corn plants, 62 percent of the borers were still on the plant on which they had hatched. Table I shows 62.5 percent recovery from artificial corn plants under sunlight conditions. The observation of Neiswander et al. (1928a) that the first instar larvae leaving the plant did so by spinning down on silken webs from the tips and edges of the leaves was also true of the behavior of the larvae on the artificial plants. Neiswander *et al.* (1928b) found that, prior to the beginning elongation of the tassel, all borer larvae on the corn plant were to be found behind leaves and within the roll of unexpanded whorl leaves. Table I shows that the borer larvae recovered from artificial plants in sunlight were also exclutaxis is indicated by the field observations of Neiswander (1928a) and other workers. Mature fifth instar larvae, however, display a change in behavior and may leave the host plant and migrate before pupation (Barber 1924, Caffrey and Worthley 1927, Hodgson 1928, Neiswander *et al.* 1928a, Neiswander and Polivka 1931, Neiswander and Savage 1931). Since such behavior does not occur until after the larvae have completed their feeding, this tendency is not within the scope of the present study.

CHEMICAL ATTRACTANTS

The experiments with artificial corn plants indicated that the initial distribution of the larvae could be accounted for on the basis of their reactions to light and contact pressure. The possibility remained, however, that chemical attractant factors might play a role in larval orientation and establishment, and might determine the feeding site of the borers in situations where their reactions to light and pressure were satisfied. Beard (1941) stated that the tassel is very attractive to young borer larvae during

one stage of plant growth and that the attractiveness of the tassel is superseded by that of the developing ear at a later stage. Several other workers have also commented upon the apparent attractiveness of different parts of the corn plant to borer larvae.

In an attempt to determine the relative attractiveness of different parts of the corn plant, a series of two-choice feeding reaction experiments was run in which the agar-based diets offered the newly-hatched larvae differed only in the type of plant tissue incorporated into the diets. The results obtained are presented in Table II. These data indicate that all the plant parts tested had a positive influence on larval establishment, for greater establishment was observed on any diet containing plant tissue than on the control diets. The results also show, however, that the plant parts tested were of approximately equal effect on the feeding reactions of the borer larvae. It should be pointed out that these experiments did not allow a distinction to be made between the effects of chemical attractants and feeding stimulants; only the total effect on establishment was measured. The diets employed all contained an amount of sugar (glucose) sufficient to nullify any effect that the sugar of the added plant tissue might have had on the feeding behavior of the larvae. The data show quite clearly that, under the conditions of the experiments, all of the corn plant tissues tested had approximately equal effects on larval establishment, and that no differences were found which could explain the differential feeding of the borer on the tissues of the corn plant.

TABLE II

THE EFFECTS OF DIFFERENT TISSUES OF THE CORN PLANT ON THE ESTABLISHMENT BEHAVIOR OF NEWLY-HATCHED EUROPEAN CORN BORER LARVAE
(TWO-CHOICE EXPERIMENTS)

Dietary Choice Experiment	Dietary Adjuvant		Average 24 hour larval establishment	Statistical Significance
	Plant tissue	Amount used		
(No.)		(mg/g diet)	(%)	(F)
1.	none	0	25.2	96.77**
	leaves	25	74.8	
2.	none	0	32.8	52.81**
	green tassel	25	67.2	
3.	leaves	25	44.6	4.33
	green tassel	25	55.4	
4.	leaf sheaths	25	46.9	2.10
	leaves	25	53.1	
5.	none	0	32.5	27.98**
	green silks	25	67.5	
6.	none	0	35.0	55.96**
	ear husks	25	65.0	
7.	green silks	25	52.8	1.04
	ear husks	25	47.2	

**Difference highly significant.

SACCHAROTROPHISM

A number of workers have considered the possible importance of dietary sugar in the developmental history of the European corn borer. Caffrey and Worthley (1927) reported that the borer larva feeding within the corn stalk "appears to prefer as food the portions of the stalk which are most succulent and rich in sugar." Baugh (1928) and Turner (1951) sought, but could not find, a correlation between sugar concentration in corn plant varieties and their suitability as hosts for borer larvae. Beck and Lilly (1949) found that the survival and growth of first instar borers on small corn plants were greatly improved when the host plants were supplemented with either glucose or sucrose. Batchelder (1949) speculated that carbohydrate: nitrogen ratios in the host plant influenced the location of the borer larvae on the plant. Beck (1956) found dietary sugar to have a marked influence on larval feeding behavior; it was observed that newly-hatched borer larvae which were given a choice of purified diets tended to "select" the diet containing the highest concentration of sugar. The predilection of the larvae to feed on diets containing high sugar concentrations may, for the sake of convenience, be termed saccharotrophism.

TABLE III

EFFECT OF CONCENTRATION OF DIETARY SUGARS ON THE ESTABLISHMENT RESPONSE OF NEWLY HATCHED EUROPEAN CORN BORER LARVAE
(FOUR CHOICE EXPERIMENTS)

Exp.	Sugar	Larval distribution at indicated Sugar level			
		0.18 mg/g (%)	0.54 mg/g (%)	1.62 mg/g (%)	4.86 mg/g (%)
1	Glucose	9.0	15.0	21.5	54.5
2	Fructose	11.1	19.2	23.2	46.5
3	Sucrose	10.4	19.1	25.9	44.6
		0.35 mg/g (%)	1.05 mg/g (%)	3.15 mg/g (%)	9.45 mg/g (%)
4	Glucose	13.0	17.3	24.0	45.7
5	Fructose	9.5	18.0	29.6	42.9
6	Sucrose	14.5	18.2	27.3	40.0
		0.75 mg/g (%)	2.25 mg/g (%)	6.75 mg/g (%)	20.25 mg/g (%)
7	Glucose	8.7	11.6	29.0	50.7
8	Fructose	8.6	16.2	27.3	47.9
9	Sucrose	9.4	16.9	28.8	44.9

The possible importance of saccharotrophism

in the feeding habits of the borer on the corn plant has been studied. A series of experiments was run to determine some of the characteristics of the saccharotrophic response. Table III shows a comparison of the larval response to glucose, fructose, and sucrose. These data demonstrate a saccharoptrophic response to the sugars tested and show that the responses to the different sugars were essentially similar over the concentration ranges employed. The results also show that the larval response was not to the absolute quantities of sugar present but rather to the relative differences in concentration among the diets available in the experimental array. This was found to be true also well beyond the concentration range shown in Table III; the relationship appears to hold at least 60 milligrams of sugar per gram of diet. In a previous paper (Beck 1956) it has been shown that the larval response thresholds for these three sugars are different and that the larval sensitivity to concentration differences is dependent on the concentrations employed. Within the range of sugar concentrations likely to be encountered in corn plant tissue, there appears little reason to attempt to distinguish the responses to the different sugars. Therefore, in analyses of plant tissues, given below, only total sugars were estimated.

Since, within a broad range of concentrations (0.2 to 60 mg/g), the larval response was to the relative sugar concentrations, any influence of plant tissue sugar on borer feeding sites must be in the form of relative differences in sugar content between adjacent plant parts in the areas of the plant satisfying the borer's phototactic and thigmotactic responses. The term ***borer choice situation*** is used below to describe situations on the host plant in which the borer larva may feed on either of two or more tissues without migrating to different parts of the plant. Thus, a borer that has become situated behind a leaf sheath may feed on either leaf sheath or stem tissue; here the borer choice situation involves only these two tissues. Borer choice situations may be described involving other groups of plant tissues, such as parts of developing ears, parts of the whorl, the developing tassel and its wrapping leaves, etc. Sugar analyses were run on plant parts from borer choice situations, where field observations have clearly indicated the tissue on which the borer feeds most extensively. The results are shown in Table IV.

The analytical data and field observations of the borer's principal feeding sites demonstrate a well-defined tendency for the borers to concentrate their feeding on the plant part containing the largest amount of sugar. Much of the behavior of the borer on the plant can be explained on the basis of its feeding response to sugar. It is a well established observation that young borer larvae tend to feed deep in the whorl of corn plants in a pre-tasselling growth stage. Batchelder (1949) observed that the first instar larvae situated within the whorl of the host plant tended to feed just below the upper margin of the film of moisture which formed between adjacent leaves. Under dry weather conditions, the film of moisture tended to move down deeper into the whorl and was accompanied by the feeding borers. It was not determined, however, whether the water acted only as a barrier to borer penetration or elicted a positive hydrotactic response from the larvae. Borer choice situation 2, Table IV, shows that a rather steep sugar gradient exists in the leaves of the whorl, and the tendency for the larvae to feed downward toward the base of the whorl can be accounted for on the basis of their reaction to light, contact, and sugars. Although a hydrotactic response has been described for newly-hatched borer larvae (Chin 1951 and Beck 1956), instars later than the first were found to be indifferent to the humidity gradients likely to be encountered on the corn plant (Chin 1951). The hydrotaxis of the newly-hatched larvae probably has but a minor influence on the orientation of the borer on the host plant.

Analyses of the sugar content of the inner husks and silks of young ears of corn showed high sugar levels with but little difference between the two tissues. In the early development of the ear, borer larvae will tend to feed on the silks in preference to the husks (Table IV, situations 16, 17, 19, 20). Since the sugar levels are nearly the same and chemical attractants are probably not involved (Table II), feeding preference may be on the basis of the physical characteristics of the tissue, and the very young larvae feed on the silk tissue in preference to the more fibrous husks. The physical characteristics of the plant tissues probably influence the feeding habits of very young larvae—(as in the inability of first instar larvae to penetrate the stalk)—but this influence becomes less important as the borers grow larger, and in the over-all relationship between the borer and the corn plant it is probably of only minor importance.

A number of workers have reported that first instar larvae of the mid-summer generation may feed on pollen that has accumulated in the leaf axils (Cox 1955, and others). In the present study, leaf tissue at the axil was found to contain about 9 mg. of sugars per gram of tissue. No sugar analyses were made of pollen, but Haber and Gaessler (1942) found 52 mg. of sugar per gram of corn pollen. The pollen-feeding habit is, therefore, consistent with the hypothesis that the feeding sites are determined by the sugar contents of the plant tissues.

Borer larvae established on small corn plants feed on leaf tissue deep in the whorl. As the tassel elongates, the borers tend to invade it and feed there; this shift in feeding behavior is apparently the result of the plant's translocation

of sugars into the tassel for pollen development, (Table IV, situation 5). When the tassel unfurls, most of the larvae are exposed and unprotected, and despite the high sugar content of the tassel tissue, they move to lower parts of the plant. They may enter any of the number of situations described in Table IV, in satisfaction of their reactions to light and contact.

TABLE IV

PLANT TISSUE SUGAR LEVEL AND PRINCIPAL FEEDING SITES OF THE EUROPEAN CORN BORER ON THE CORN PLANT

	Borer choice situation	Tissue Total Sugars	Observed Principal feeding site
		(mg/g fresh wt.)	
	Plant stage: whorl, 36 inches extended height		
1.	Base of ligulated leaf	4.5	
	Outer leaves of whorl	11.8	Outer leaves of whorl
	Distal portion, whorl leaves	6.6	
2.	Middle portion, whorl leaves	11.4	
	Basal Portion, whorl leaves	19.2	Basal portion, whorl leaves
3.	Inner whorl leaves	11.1	
	Outer whorl leaves	11.8	Both tissues
4.	Base of leaf	4.5	
	Leaf sheath	8.8	Leaf sheath
	Plant stage: late whorl, 55 inches extended height		
5.	Terminal leaf enclosing tassel	18.3	
	Green tassel	31.4	Green tassel
	Plant stage: very young ear, no external silk		
6.	Sheath around ear shoot	13.5	
	Outer husks of ear shoot	19.7	Outer husks
7.	Base of leaf adjacent to ear shoot	8.5	
	Tip of outer husks of ear shoot	16.3	Outer husks
8.	Leaf sheath around ear shoot	13.5	
	Shank of ear shoot	21.7	Ear shank
9.	Base of outer flag leaves	10.1	
	Tip of outer husks	16.3	Tip of outer husks
10.	Base of rolled flag leaves	21.1	
	Enclosed silks	32.8	Enclosed silks
11.	Outer husks of ear shoot	18.0	
	Inner husks of ear shoot	25.7	Inner husks
12.	Inner husks of ear shoot	25.5	
	Developing ear	31.4	
	Silks around developing ear	38.7	Silks
	Plant stage: Exposed green silk		
13.	Leaf sheath	22.4	
	Ear shank	31.2	Both internode and ear shank
	Internode	33.8	
14.	Leaf sheath	20.5	
	Outer husk	31.2	Outer husk
15.	Base of flag leaves enclosing silks	28.9	
	Silks enclosed by flag leaves	36.8	Silks
16.	Tip of outer husks	29.9	
	Tip of inner husks	38.0	
	Silks at husk tips	40.9	Silks
17.	Developing ear	30.8	
	Inner husks	40.5	
	Silks at ear	40.9	Silks
	Plant stage: Exposed brown silks, kernels between blister and milk stage		
18.	Leaf sheath	27.8	
	Ear shank	36.0	
	Internode	43.2	Both internode and ear shank
19.	Tip of outer husks	32.2	
	Tip of inner husks	37.5	
	Silks enclosed by husks	39.9	Silks
20.	Kernels	28.8	
	Inner husks	37.0	
	Enclosed silks	41.0	Silks

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SUMMARY

The experimental results presented indicate that the orientation and feeding behavior of European corn borer larvae on the corn plant can be accounted for on the basis of the negative phototactic, positive thigmotactic, and saccharotrophic behavior responses of the larvae. Chemical attractants appear to play no role in the determination of borer feeding sites. The physical characteristics of the plant tissues, however, have at least a minor influence on feeding behavior of early instar larvae. The initial distribution of the larvae on the plant is the result of the borer's negative phototaxis and positive thigmotaxis. Within the parts of the plant invaded by the borer in satisfying its reaction to light and contact pressure, it has a choice of plant tissues on which to feed. Of the plant parts available to it, the borer tends to concentrate its feeding on the tissues containing the highest levels of sugar.

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SYNOPSIS OF THE ECONOMIC SPECIES OF TROGODERMA OCCURRING IN THE UNITED STATES WITH DESCRIPTION OF A NEW SPECIES (COLEOPTERA: DERMESTIDAE)[1]

R. S. BEAL, JR.[2]

The recent introduction into the United States of the Khapra beetle, *Trogoderma granarium* Everts, a serious pest of granaries, necessitates a review of those species with which it might be confused and requires a revision of the existing keys to the nearctic species of the genus. Although the species is adequately described by Hinton (1945) and others, it is difficult to separate from some other nearctic species. Particularly is this true of its immature stages. My hope is that the keys and discussion which follow will simplify its identification, particularly for the worker not specializing in the taxonomy of the group.

A second purpose of this paper is to make available some additional information on the taxonomy of the various members of the genus. It is necessary, for one thing, to correct some nomenclatural errors made in my previous revision of the nearctic species of the group (1954). At the time of the writing of this earlier paper, information was not available on the identity of some of the Old World *Trogoderma*. Since then specimens of the Palearctic species in question have been made available to me and the need for several changes has been pointed out. It is, of course, annoying to the non-taxonomist when changes are made in the names of well-known, economically important species. However, the corrections made here will help eliminate some of the confusion which exists in descriptions of the species and will help clear the way for future progress in understanding the biology of the various forms.

A third purpose is to indicate geographical distributions, foods and food preferences, and other details of general biological interest pertaining to these species. The included lists of stored products in which several of the species have been found have been compiled for the most part by F. L. Blanc and G. T. Okumura of the Insect Identification Laboratory of the California Bureau of Entomology.

A number of workers deserve my best thanks for having contributed the results of their own studies on the group, for having given of their time in the inspection of type specimens, and for having contributed material used in this study. Among them particular mention should be made of W. H. Anderson of the Entomology Research Branch of the United States Department of Agriculture, J. Blafour-Browne of the British Museum, Vladimír Kalík of Pardubice, Czechoslovakia, Maciej Mroczkowski of the Polish Academy of Sciences, M. W. Sanderson of the Illinois Natural History Survey. To R. W. Howe and H. D. Burges of the Department of Scientific and Industrial Research of England I am particularly grateful for permission to use data from a paper of theirs which, at this writing, is still in press. For a critical reading of the manuscript and for a large body of data incorporated in the paper I am indebted to F. L. Blanc and G. T. Okumura. Finally I wish to thank Mr. W. P. Wilkin for the use of optical equipment employed in this and in other studies.

Adults of *Trogoderma* may be separated from all other described Nearctic genera of dermestid beetles by the following combination of characters. (1) The setae of the dorsal surface are not strongly scale-like, although the light-colored hairs may be somewhat flattened and ensiform. (2) The head bears a median ocellus. (3) The club of the antenna is composed of at least three segments, and the terminal segment is never greatly enlarged, but is subequal in length to the preceding two or three segments. (4) The posterior oblique margin of the antennal cavity is elevated and more or less carinate at least as far as the middle of the cavity. Hence the antennal cavity is at least partly enclosed behind. (5) The basal segment of each hind tarsus is as long or longer than the second segment, never shorter.

KEY TO ADULTS OF NEARCTIC SPECIES OF TROGODERMA (INCLUDING FORMS NOT KNOWN TO BE OF ECONOMIC IMPORTANCE)

1. Dorsal pubescence unicolorous (rare species not known to occur in stored products) 2
— Hairs of dorsum of two or three colors. (The dorsal pubescence of *granarium* and a few other species is easily rubbed off. Any completely abraded specimen probably falls in this half of the couplet) 3

2 (1). Elytral pubescence closely appressed; female antennal club compact; segments of antenna only very slightly eccentric in male, not at all eccentric in female (Northern States and Canada) ..... **sinistrum** Fall
— Elytral pubescence subdecumbent; female antennal club not compact, pedicels more or less visible; male antenna flabellate; female antenna pectinate (Southern Arizona) ..... **ballfinchae** Beal

3 (1). Elytra with a single antemedian band of light maculation, base of elytra rarely tending to

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be somewhat light as well (rare species not known to occur in stored products)..... 4

— Elytra with more than one band of light maculation, or with no bands of light maculation, or with a pattern of three light bands indicated by areas of light pubescence..... 5

4 (3). Male antennae strongly flabellate, female antenna pectinate; punctures on disc of pronotum separated by one-fourth to three-fourths diameter; antennal fossa completely margined behind by knifelike carina (Florida)... ..... **fasciferum** Blatchley

— Segments of antennae at most slightly eccentrically placed on pedicels; punctures on disc of pronotum contiguous; antennal fossa margined behind by a low, subacute ridge along median third, indistinctly margined along lateral two-thirds (Texas and Oklahoma)..... **primum** (Jayne)

5 (3). Form long and narrow, ratio of width (across base of elytra) to length (of pronotum and elytra) greater than 1:2.1; antennal fossa with low and subacute carina on median two-thirds of posterior diagonal margin, open behind on lateral third; light colored hairs of elytral fasciae almost entirely white: less than three percent light golden brown..... ..... **angustum** (Solier)

— Form relatively shorter, ratio of width to length 1:2.0 or wider; antennal fossa more or less completely closed behind by acute posterior diagonal carina; light colored hairs of elytral fasciae only rarely predominately white.... 6

6 (5). Antennal cavity coarsely and confluently punctate on all surfaces except for small smooth area near prosternal suture, punctures two or three times as large as facets of eye; female antennal club 5- or 6-segmented..... ..... **simplex** Jayne

— Antennal cavity finely punctate with punctures about as fine as facets of eye, or shining and minutely striate; female antennal club 3- or 4-segmented with seventh segment of antenna somewhat enlarged or not..... 7

7 (6). Elytral cuticle unicolorous or vaguely mottled without a clearly defined pattern (at least in the nearctic forms); number of antennal segments varying from nine to eleven; male antennal club with five segments at most (fig. 1); female antennal club with three segments (fig. 1)..... **granarium** Everts

— Elytra with a definite pattern (although in some species, as *ornatum*, the pattern may be fine and intricate), the pattern visible either in the maculation or in the pubescence or both; antenna always of eleven segments; male antennal club of at least five segments; female antennal club of at least four segments (fig. 2)..... 8

8 (7). Integument unicolorous, black (or dark brown in teneral specimens), or black with vague brownish maculations on the humeri and apical elytral margins only, never with basal, submedian, and subapical bands indicated in the maculation (these indicated by areas of light pubescence only)..... 9

— Integument bicolorous, at least a pattern of basal, submedian, and subapical bands indicated by lighter areas in the maculation..10

9 (8). Male antennal club with segments rather loosely joined and serrate, *i.e.*, each segment eccentrically placed on pedicels (similar to *sternale*, fig. 4); surface of antennal cavity of male microgranulate; ratio of width (across base of elytra) to length (of pronotum and elytra) ranging from 1:1.81 to 1:1.98 (rare species not known to occur in stored products; coastal Northern California)..... ..... **paralia** Beal

— Male antennal club compact and symmetrical (fig. 3); surface of antennal cavity of male glabrous and shining, obscurely marked with oblique striae; ratio of width (across base of elytra) to length (of pronotum and elytra) ranging from 1:1.60 to 1:1.75..... ..... **glabrum** (Herbst)

10 (8). Area of light maculation of elytra not extending to base of elytra, but separated from base by at least twice length of scutellum..... ..... **grassmani** Beal

— Area of light maculation of elytra extending entirely to base of elytra, or separated from base by not more than one-half length of scutellum.....11

11 (10). Inner margin of eye distinctly emarginate.... ..... **inclusum** LeConte

— Inner margin of eye straight or very slightly sinuate.....12

12 (11). Male antennal club distinctly serrate with segments loosely joined so that pedicels are visible; basal band or "loop" of elytral maculation always connected with submedian elytral band by longitudinal lines of light maculation, the longitudinal lines occasionally so broad that basal light maculate area appears fused with submedian band of light maculation.....13

— Male antennal club not serrate, terminal segments always more or less compactly joined; elytron with basal light maculate band or "loop," submedian band, and apical band well marked but without distinct longitudinal lines of light maculation connecting these bands (in *parabile* traces of longitudinal lines may rarely show around the submedian band, but these do not actually connect with the basal band or "loop.").....14

13 (12). Third segment of male antenna approximating second and fourth segments in length and width; segments of male antennal club decidedly eccentric or pectinate; elytral pattern of light maculation consisting of thin lines, the basal "loop" of light maculation bisected by a thin longitudinal line of light maculation ..... **ornatum** Say

— Third segment of male antenna minute, in length and width about half either second or fourth segments; segments of club only moderately eccentric (fig. 4); elytral pattern of light maculation consisting of either broad or thin lines, but if of thin lines, then the basal "loop" of light maculation not bisected by a longitudinal line of light maculation... ..... **sternale** Jayne

14 (12). Light hairs of pronotum almost entirely golden yellow; antennal club of male 8-segmented; eleventh segment bluntly rounded at apex.. ..... **parabile** Beal

— Light hairs of pronotum consisting of at least one third white hairs; male antennal club 5- or 6-segmented; eleventh segment acutely pointed at apex..... **teukton** n. sp.

Larvae of species of the tribe Megatomini, to which *Trogoderma* belongs, may be distinguished by the following combination of characters. (1) Hastisetae (spear-headed hairs) are present on the abdominal tergites. (2) The tufts of hastisetae arise from the sclerotized dorsal surface of the abdominal tergites. (3) The tufts of hastisetae do not obviously converge over the cauda. It is a great deal more difficult to separate larvae of genera within the Megatomini. However, since it is quite unusual to find Nearctic species of such genera as *Megatoma* and *Globicornis* as

economic pests, the separation of their larval forms from *Trogoderma* will not be considered here. Details of the larval characters of these genera, so far as they are known, will be found in Rees (1943). One species, the parthenogenetic *Perimegatoma vespulae* Milliron, is an occasional pest of insect collections in Northern California and has been found in San Mateo County as a house pest. On this account it is included in the key to the larvae.

KEY TO KNOWN LARVAE[3]

1. Acrotergites without setae except for two or four very short, smooth, sensory setae near posterior margins..... **Perimegatoma vespulae** Milliron
— Acrotergites with numerous, fine spicisetae (spinulate or "rat-tail" setae) in addition to the two or four, short, sensory setae..... 2
2 (1). Setae of basal segment of antenna less than half as long as second segment of antenna; each tergite bearing numerous, short, somewhat blunt, inwardly directed spicisetae, which are about twice as thick as spicisetae of acrotergite, (fig. 5)..... **Trogoderma simplex** Jayne
— Setae of basal segment of antenna at least three-fourths as long as second segment of antenna; tergites without such spicisetae as those described above..... 3
3 (2). One or more setae present on second segment of antenna..... 4
— No setae present on second segment of antenna.. 5
4 (3). Seventh and eighth abdominal tergites each with a distinct antecostal suture (transverse line near anterior margin of sclerotized area) (similar to *grassmani*, fig. 6)..... **sternale** subspecies
— Seventh abdominal tergite with suture faint or interrupted; eighth abdominal tergite with suture entirely absent or (rarely) present but interrupted (fig. 7)..... **granarium** Everts
5 (3). Setae of acrotergites all long enough to extend across antecostal suture. If setae do not extend across much more than anterior third of tergite, and if occasionally a very few setae are not long enough to extend across antecostal suture, then the sensory pores of the third antennal segment will be found distad to the basal third of the segment..... 6
— Anteriormost setae of acrotergites not extending across the antecostal suture. If this is questionable, only a very few setae not extending across antecostal suture, then at least one sensory pore of the third antennal segment will be found in the basal fourth of the segment..... 7
6 (5). Antecostal sutures of seventh and eighth abdominal tergites extending completely across the tergites (fig. 6); basal sensory pores of third antennal segment situated at about basal fourth..... **grassmani** Beal
— Antecostal sutures of seventh and eighth abdominal tergites not extending completely across the tergites, suture of eighth segment sometimes entirely absent; basal sensory pores of terminal antennal segment situated distad of basal third..... **ornatum** (Say)
7 (5). Antecostal suture of eighth abdominal tergite absent or (rarely) weak and interrupted at several points..... **granarium** Everts
— Antecostal suture of eighth abdominal tergite present and uninterrupted except occasionally at midline..... 8
8 (7). Setae of basal antennal segment arranged in a whorl, almost completely encircling the segment: setae not bunched on mesal side of the segment (fig. 8a)..... 9
— Setae of basal antennal segment bunched on mesal side of segment, one third or more of the outer portion of the segment bare (fig. 8b).... **parabile** Beal
9 (8). Integument yellowish or cream-colored..... **inclusum** LeConte
— Integument medium to dark brown (except in teneral forms)..... **glabrum** (Herbst)

[3]Because of a lack of material for an adequate study of the varietal differences, *Trogoderma angustum* (Solier) has not been included in the key. A description of this species, insofar as it is known, will be found in Beal (1954).

DISCUSSION OF SPECIES[4]

Trogoderma angustum (Solier)

Eurhopalus angustus Solier, 1849–1851, *in* Gay, Historia Fisica y Politica de Chile, Zoologia, 4: 374.
Trogoderma angustum, Beal, 1954, Univ. of Calif. Pub. in Entom., 10(2): 55–56.

A Chilean species, recorded in the United States from Ithaca, New York, Lewiston, Maine, and San Francisco, California. There is no evidence that it has yet become established at any of these localities. It is known to infest stored grain, and is found as a pest of insect collections.

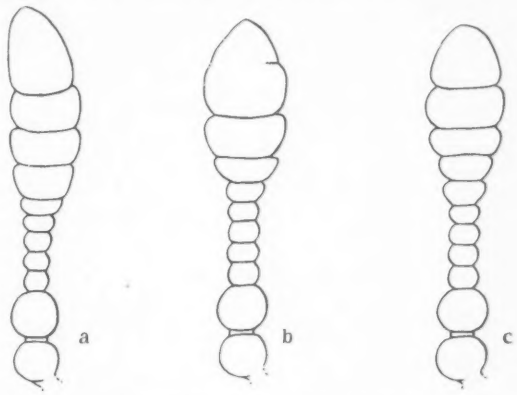

FIG. 1. Antennae of *T. granarium:* (a) male antenna with normal number of segments; (b) female antenna with reduced number of segments; (c) female antenna with normal number of segments.

Trogoderma glabrum (Herbst)

Anthrenus glaber Herbst, 1784, *in* Füessly, Arch. Ins., 4: 26.
Anthrenus niger Herbst, 1797, *in* Jablonsky, Nat. Ins. (Käfer), 7: 338, pl. 115, fig. 2.
Trogoderma nigrum, Mulsant and Rey, 1867 (1868), Ann. Soc. Linn. Lyon (n.s.), 15: 122; Ganglbauer, 1904, Käf. Mitteleur., 4(1): 35.
Trogoderma nigrescans, Hicks, 1953, Jour. Kans. Ent. Soc., 26: 13, 14.
Trogoderma boron Beal, 1954, Univ. of Calif. Pub. in Entom., 10(2): 80–83; Chao, 1954, Pan-Pac. Ent., 30: 260–262 (*new synonymy*).

[4]The synonymical bibliographies which follow are not intended to be complete. Where nomenclatural changes are made, references are given where the author is reasonably certain of the identity of the species treated. Otherwise references are given only to the original description, to the latest revision, and to subsequent biological treatments.

There is little question but that *Trogoderma boron* Beal is identical with the Palearctic *glabrum* (Herbst). Mr. Maciej Mroczkowski of the Polish Academy of Sciences has very kindly examined the types of Herbst's species, which are in the Zoological Museum in Berlin, and has found them to be similar in every significant external character. A series of European specimens furnished by Mr. Mroczkowski seems also to fall within the range of variation of our Nearctic specimens. It is uncertain whether the form treated by Hinton (1945) under this name is conspecific. In some respects Hinton's description seems to apply to *teukton*, a species described in this paper as new.

Glabrum is commonly found as a pest in granaries. That it will live on cereal food alone has been verified by maintaining a culture of the species for several generations on yellow cornmeal. Unlike *granarium*, however, populations of *glabrum* do not seem to build up to

FIG. 2. Female antenna of *T. inclusum*.

FIG. 3. Male antenna of *T. glabrum*.

FIG. 4. Male antenna of *T. sternale maderae*.

immense proportions on a diet of grain alone. In nature the species is usually found feeding on dried insect remains (Beal, 1954). Recently it has been found in California feeding on "dehydrated meal" and on soybean meal.

The species has been recorded in the United States from Colorado, Idaho, Illinois, Iowa, Kansas, Minnesota, Missouri, Oregon, and Washington. Additional records are the following: CALIFORNIA: Solano County, Santa Barbara County, Kern County: WYOMING: Platte County (D. G. Denning).

Trogoderma granarium Everts

Trogoderma granarium Everts, 1899, Coleop. Neerland's, 1: 610; Hinton, 1945, Beetles assoc. with stored prod., 1: 387–395; Howe, 1952, Bull. Ent. Research, 43(1): 111–144; Howe and Burges (in press).

The Khapra beetle, one of the most serious of all storage pests, is a native of India, Ceylon, and Malaya. It is thoroughly established in nearly all parts of the Old World, including Australia and Africa. In 1953 it was first identified in the United States from warehouses in Tulare County, California, but apparently it was present in a warehouse in Fresno, California, as far back as 1946 (Special Report of the Joint Interim Committee on Agricultural and Livestock Problems, 1955). As of April 1, 1955, it had spread to sixteen California counties, six Arizona counties, two New Mexico counties, and to Baja California, Mexico. In October, 1955, it was known to occur in one additional California county, but apparently had been eradicated in Mohave county, Arizona, and in Kings, Madera, and Sutter counties, California. Its present known distribution by counties is as follows: ARIZONA: Graham, Maricopa, Pima, Pinal, Yuma; CALIFORNIA: Alameda, Butte, Colusa, Fresno, Glenn, Imperial, Kern, Lake, Los Angles, Riverside, San Bernardino, San Diego, San Francisco, Tulare; NEW MEXICO: Curry, Roosevelt.

A summary of work being done on the economic control of the species in California will be found in the Special Report of the Joint Interim Committee on Agricultural and Livestock Problems (1955). Howe (1952) has an excellent study of the food storage problem created by the Khapra beetle. Hinton (1945) gives a description of the species and a summary of its biology.

Trogoderma grassmani Beal

Trogoderma grassmani Beal, 1954, Univ. of Calif. Pub. in Entom., 10(2): 77–79.

This species is recorded from Arizona, Imperial, Inyo, Kern, and Riverside counties in California, Southern Utah, and Lerdo, Durango, Mexico. Additional localities on the periphery of its known distribution are the following: BAJA CALIFORNIA, MEXICO: 20 miles west of Mexicali, (C. D. Michener); UTAH: Richfield, St. George, (E. W. Davis); and the following counties in CALIFORNIA: Glenn, Kings, Los Angeles, Madera, Merced, Monterey, San Joaquin, Stanislaus, Tulare.

Recent surveys in California by the state and federal governments have found it as a pest in the following stored products: barley, fish meal, beet seed, alfalfa seed, cotton seed, flax seed, clover seed, and candy.

Trogoderma inclusum LeConte

Trogoderma inclusum LeConte, 1854, Proc. Acad. Nat. Sci., Phila., 7: 110; Jayne, 1882, Proc. Amer. Philos. Soc., 20: 363; Casey, 1900, Jour. N. Y. Ent. Soc., 8: 154; Casey, 1916, Memoirs on the Col., 7: 186.

Trogoderma tarsale (non Melsheimer, 1846), Riley, 1894, Ins. Life, 6: 215; Wodsedalek, 1913, Jour. Anim. Behav., 3:61; Wodsedalek, 1917, Science, 46: 366.

Trogoderma advena Casey, 1900, Jour. N. Y. Ent. Soc., 8: 154.

Trogoderma obsolescens Casey, 1900, Jour. N. Y. Ent. Soc., 8: 154; Casey, 1916, Memoirs on the Col., 7: 185.

Trogoderma nigrescans Casey, 1916, Memoirs on the Col., 7: 189.

Trogoderma frosti Casey, 1916, Memoirs on the Col., 7: 188.

Trogoderma versicolor (non Creutzer, 1799), Mutchler and Weiss, 1927, N. J. Dept. Agric. Cir., 108: 23; Hinton, 1945, Beetles assoc. with stored prod., 1: 377; Howe, 1952, Ent. Monthly Mag., 88: 182; Beal, 1954, Univ. of Calif. Pub. in Entom., 10(2): 83–85.

Two apparently distinct species were synonymized by Arrow (1915) under the name *versicolor*. One form occurs on the continent of Europe, the other in North America and the British Isles. The name *versicolor* (Creutzer) should be applied to the European form and *inclusum* LeConte to the American and British form. The former is the species indicated in the faunistic list of Dermestidae in Czechoslovakia (Kalík, 1948) and in Poland (Mroczkowski, 1954). The latter is the form which has been described by Hinton (1945) and by Howe and Burges (in press). According to J. Balfour-Browne (personal communication) all the specimens in the British Museum from the British Isles are of the *inclusum* type. Whether *inclusum* was originally indigenous to England or to North America will probably never be known.

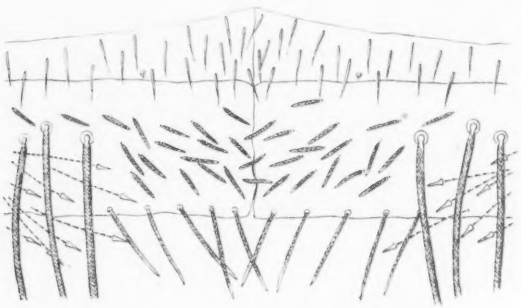

FIG. 5. Median area of first abdominal tergite of mature larva of *T. simplex*.

Trogoderma inclusum may be distinguished from *versicolor* by the following characters. The eyes bear a definite medial emargination which is entirely lacking in *versicolor*. The floor of the male antennal cavity of *inclusum* is finely striate and shining, whereas in *versicolor* it is microgranulate and dull. The first abdominal sternite of *inclusum* has fine, long or short striae extending obliquely outward from the inner margins of the metacoxal cavities. These are wanting in *versicolor*. In addition to these external characters the male genitalia show seemingly constant differences, particularly in the width and shape of the "bridge" of the phallobase (figure 9).

This species has been recorded from the following states: Arizona, California, Connecticut, District of Columbia, Georgia, Idaho, Illinois, Indiana, Iowa, Kansas, Maryland, Massachusetts, Minnesota, Mississippi, New Hampshire, New Jersey, New York, North Carolina, Ohio, Oklahoma, Pennsylvania, South Dakota, Tennessee, Texas, and Utah. Records for states from which the species has previously been unreported are the following: ARKANSAS: Washington County, (L. H. Rolston); LOUISIANA: Baton Rouge, (Wickham); MISSOURI: Columbia, (W. S. Craig); OREGON: Eugene, (B. Malkin); WYOMING: Laramie, (H. Sweetman).

Although *inclusum* is often found in grain storage, it has not been established that it can subsist on grain alone. It is a common pest in dried milk factories, and is often found infesting a wide range of other dried protein substances. In California it has been found infesting a bee hive.

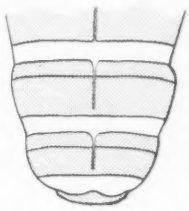

FIG. 6. Seventh and eighth larval abdominal tergites (denuded) of *T. grassmani*.

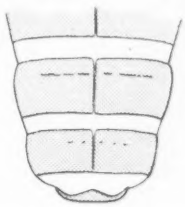

FIG. 7. Seventh and eighth larval abdominal tergites (denuded) of *T. granarium*.

Trogoderma ornatum (Say)

Megatoma ornata Say, 1825, Jour. Acad. Nat. Sci. Phila., 5: 186.

Trogoderma ornatum, Beal, 1954, Univ. of Calif. Pub. in Entom., 10(2): 66–69.

Ornatum has been recorded from the following states: California, Connecticut, District of Columbia, Illinois, Iowa, Kansas, Maryland, Massachusetts, Minnesota, Missouri, New Hampshire, New Jersey, New York, Ohio, Ontario, Pennsylvania, South Carolina, Tennessee, Texas, and Virginia. Records for states from which the species has previously been unreported are the following: LOUISIANA: Harahan, (F. G. Werner); NORTH CAROLINA: Black Mountains, (Van Dyke collection); SOUTH CAROLINA: Charleston, (Hubbard and Schwarz); VIRGINIA: Afton, (Hubbard and Schwarz). The species has previously been reported from only one locality each in California and Texas. These localities are rather widely separated from the area of natural distribution of the species. Hence records of distributions from these states are of interest.

California counties in which the species has been found to occur are the following: Alameda, Imperial, Kern, Los Angeles, Monterey, Orange, Riverside, San Luis Obispo, San Mateo, Santa Barbara. New records from Texas are the following: Austin County (D. J. and J. N. Knull); Corpus Christi (D. J. and J. N. Knull).

Trogoderma ornatum can evidently subsist on cereal foods alone. In the recent California survey it was found infesting the following stored products: beans, barley, cottonseed pellets, broom corn, beet seed, fish meal, canary grass seed, and oats. It is apparently never a serious pest of granaries.

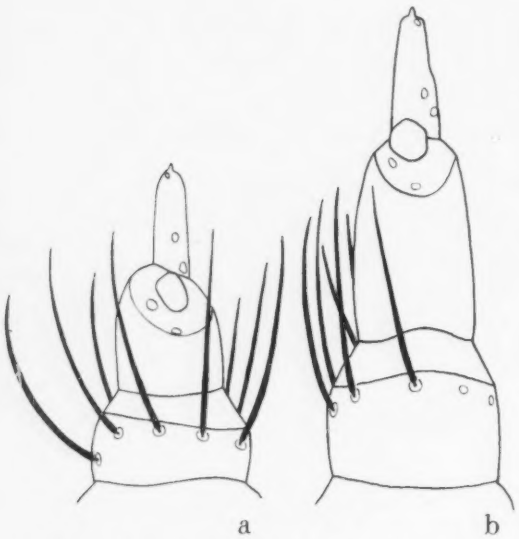

FIG. 8. Larval antennae of (a) *T. inclusum* and (b) *T. parabile*.

Trogoderma parabile Beal

Trogoderma parabile Beal, 1954, Univ. of Calif. Pub. in Entom., 10(2): 87–89.

This species has been recorded from the following states, for the most part from infestations of stored products: Arizona, California, Colorado, District of Columbia, Idaho, Minnesota, New Jersey, Oregon, Pennsylvania, and Utah. The species appears to occur much more frequently than appeared to be the case at the time of its original description. Initially recorded from only four California counties, Alameda, Fresno, Kern, and Tulare, it has subsequently been found in twenty-six others. These are the following: Butte, Calaveras, Colusa, Glenn, Imperial, Kings, Los Angeles, Madera, Merced, Modoc, Napa, Orange, Placer, Riverside, San Benito, San Bernardino, San Diego, San Joaquin, Santa Clara, Shasta, Stanislaus, Sutter, Tehama, Ventura, Yolo, and Yuba. New records outside California are the following: IOWA: Ames, (W. S. Craig); UTAH: Dog Area, Dugway Proving Ground, Tooele County, (P. D. Ashlock).

The species has been reared through several generations on yellow corn meal, establishing the fact that it can subsist on cereal products alone. It has been found in California as a pest on stored seeds of grass, spinach, cucumber, egg plant, tomato, safflower, sunflower, onion, cantaloupe, pumpkin, sugar beet, alfalfa, melon, alta fescue, millet, pepper, cotton, sour clover, and beet, as well as on such cereals as corn, barley, oats, sorghum, wheat, pop-corn, wild rice, and rice. Legumes and nuts on which it has been found are peas, lima beans, beans, walnuts, and chestnuts. It is also frequent on stored products of high protein content such as dried skim milk, fish meal, beef cubes, and almond meal. The specimens recorded above from Ames, Iowa, were found in stores of warfarin rat bait. Although it is a fairly common pest of granaries, it never seems to reach the serious proportions of *T. granarium*.

Trogoderma simplex Jayne

Trogoderma simplex Jayne, 1882, Proc. Amer. Philos. Soc., 20: 362, pl. 2, figs. 51, 52; Beal, 1954, Univ. of Calif. Pub. in Entom., 10(2): 57–64; Chao, 1954, Pan-Pac. Ent., 30: 260–262.

This species is known from every state west of the Rocky Mountains including New Mexico, Colorado, Wyoming, and Montana. It is also known from Iowa, New Jersey, New York, North Dakota, and Pennsylvania, but is probably not indigenous to these.

Flowers on which the adults have been taken include the following: cotton at Tucson, Arizona (G. D. Butler); citrus at Tucson, Arizona (F. G. Werner); *Pluchea* sp. at Yuma, Arizona (Butler and Werner); *Acacia* sp. at Sabino Canyon, Pima County, Arizona (F. G. Werner); *Heliotropum* sp. at Furnace Creek, Death Valley, California (J. W. MacSwain).

Simplex has not been considered an economically important species. However, in a survey of grain elevators in Southeastern Washington, Chao (1954) reported that in one instance, in an elevator at Pullman, *simplex* outnumbered any other insect. In all elevators studied in the area, Chao noted that *simplex* appeared more abundantly and frequently than *glabrum*.

Trogoderma sternale subspecies

Trogoderma sternale sternale Jayne, 1882, Proc. Amer. Philos. Soc., 20: 363, pl. 2, fig. 50; Beal, 1954, Univ. of Calif. Pub. in Entom., 10(2): 71.

Trogoderma sternale maderae Beal, 1954, Univ. of Calif. Pub. in Entom., 10(2): 71, 73.

Trogoderma sternale deserti Beal, 1954, Univ. of Calif. Pub. in Entom., 10(2): 73.

Trogoderma sternale aspericolle Casey, 1900, Jour. N. Y. Ent. Soc., 8: 154; Beal, 1954, Univ. of Calif. Pub. in Entom., 10(2): 74.

Trogoderma sternale complex Casey, 1900, Jour. N. Y.

Ent. Soc., 8: 153; Beal, 1954, Univ. of Calif. Pub. in Entom., 10(2): 74.

Trogoderma sternale plagifer Casey, 1916, Memoirs on the Col., 7: 187; Beal, 1954, Univ. of Calif. Pub. in Entom., 10(2): 76.

The known distribution of the various subspecies of *sternale* is indicated by Beal (1954). The species extends from Southern Mississippi across the Southwestern states north into Idaho, Washington, and British Columbia. In addition it has been found in scattered Eastern and Midwestern localities where it apparently has been introduced by commerce. Doubtlessly the species extends some distance southward into Mexico. Which of the various Mexican forms should be assigned to this species has yet to be worked out.

Localities for states from which the species has previously been unreported are the following: ARKANSAS: Washington County, (L. H. Rolston), two specimens seemingly intermediate between *sternale maderae* and *sternale complex*, possibly introduced; MISSOURI: Columbia, (W. S. Craig), three specimens of *sternale plagifer*; NEVADA: La Madre Mountains, (D. J. and J. N. Knull), one specimen of uncertain subspecific relationships; OHIO: Franklin County, (J. N. Knull), and Hocking County, (D. J. and J. N. Knull), one specimen each of *sternale plagifer*; WYOMING: Laramie, an extensive series of *sternale plagifer*.

This species is best known as a pest of insect collections. It is not infrequently found in granaries, but usually as a pest of minor importance. Where it does occur in granaries, it usually seems to be in association with other insects. Nevertheless it is known to infest a wide range of stored products. In California it has been found as a pest in stored seeds of sunflower, timothy, veldt grass, snapdragon, vetch, kohlrabi, lettuce, cucumber, tomato, prairie brome, alfalfa, beet, cantaloupe, alta fescue, sour clover, cauliflower, pepper, nasturtium, safflower, spinach, and sesbania, in dehydrated alfalfa, hay, orange peel, and flax, in legumes and nuts such as walnuts, peanuts, lima beans, peas, and pecans, in cereals such as oats, corn, sorghum, wheat, barley, and bran, and in a wide variety of miscellaneous proteinaceous materials such as animal fur, dog food, cottonseed meal, linseed meal, soybean meal, and pecan husks. No data are available on the other insects or insect remains which may have been present in these infested materials. In some instances, such as in the infestation of dehydrated alfalfa, hay, and flax, it is altogether probable that *Trogoderma sternale* could have existed only as a secondary pest.

A form apparently close to the subspecies *aspericolle* but possibly distinct has been found infesting bulk dairy feed at Escondido, California (D. F. Palmer). The same form has been taken in the Ruby Mountains near Nogales, Arizona (R. S. Beal). It may be a separate Mexican subspecies which occasionally extends north of the border.

Trogoderma teukton new species

Trogoderma glabrum (non Herbst, 1783), Beal, 1954, Univ. of Calif. Pub. in Entom., 10(2): 89–92.

Adult male.—Color of integument of head and pronotum black; integument of elytra piceous with reddish brown maculations; undersurfaces piceous; legs and antennae brown. Pubescence of dorsal surfaces coarse, subrecumbent, consisting of black, light golden brown, and white hairs; light pubescence about equally divided between golden brown and white hairs; pubescence of ventral surfaces somewhat finer, recumbent, consisting of light golden brown and cinereous hairs. Antenna 11-segmented; third segment less than half as wide as second segment; segments three to ten gradually increasing in width; segment eleven narrower than ten, a little longer than segments nine and ten combined. Eye with broad, shallow, medial sinuation. Light pubescence and light maculation of elytra forming pattern of basal loop, median band, subapical band, and apical spot; pattern without longitudinal light lines between loop and subapical band.

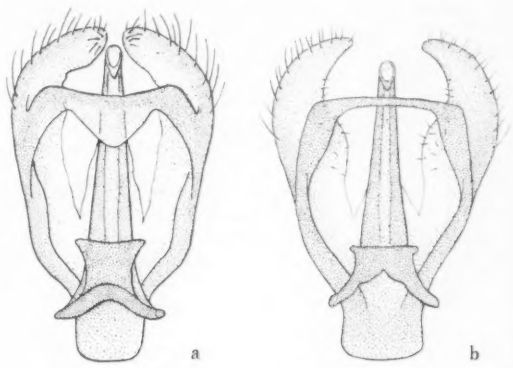

FIG. 9. Male phalli of (a) *T. versicolor* (Creutzer) and (b) *T. inclusum* LeConte.

Antennal fossa half as wide as long, extending nearly to base of prothorax, shallowly excavated; posterior oblique margin raised and extending full length of fossa as knifelike carina; floor of fossa glabrous and shining, marked with a few, fine, longitudinal striae. Metasternum marked on each side with stria extending obliquely outward from inner margin of mesocoxal cavity. First abdominal sternite marked with striae extending obliquely outward from inner margins of metacoxal cavities.

Length (of pronotum and elytra): 2.0 mm.; width (across base of elytra): 1.2 mm.

Adult female.—Antenna with third segment about half as wide as second; segments five to seven increasing gradually in width; segment eight more abruptly so, making antennal club appear 4-segmented; club widest at segment ten. Antennal fossa shallowly excavated; posterior

diagonal margin raised and carinate but carina becoming evanescent laterally; floor with microscopic, confluent punctures and fine pubescence on anterolateral half and glabrous and minutely strigate on mediposterior half.

Length (of pronotum and elytra): 2.5 mm.; width (across base of elytra): 1.4 mm.

Range of observed variations.—Color of elytra black to brown with maculations reddish brown to light brown. Light colored pubescence of dorsum varying from nearly all white hairs to nearly equal number of white and golden brown hairs. Length (of pronotum and elytra): 2.0 mm. to 2.9 mm. Ratio of width (across base of elytra) to length varying from 1:1.60 to 1:1.72.

Types.—*Holotype* male, Ramsey County, Minnesota, June 19, 1936 (H. H. Shepard); *allotype* female, same data, both deposited in the collection of the University of Minnesota; *paratypes* as follows: IOWA: (1) Sioux City (C. N. Ainslie); MINNESOTA: (4) Grey Cloud Island, October 1 and 2, 1939 (Philip Marvin); (13) Ramsey County, June 19, 1936 (H. H. Shepard); (21) St. Paul, November 30, 1938 (H. E. Milliron); December 1, 1938 (Miss Phelps); June 12, 1939 (J. Reinhardt); May 13, 1942 (M. Rockstein); NORTH DAKOTA: locality unknown (H. E. Milliron).

Beal (1954) erroneously identified this species with the European *glabrum* (Herbst). Actually *boron* Beal is a synonym of *glabrum* (Herbst). It is very probable that this species has been introduced into the United States and is identical with some previously named exotic species of *Trogoderma*. That it is an introduced species seems evident from its unnaturally limited distribution. However, on the basis of existing descriptions it is impossible to associate it with certainty with any existing name. Hence it is being described here as new.

Although very close to *glabrum*, *teukton* can be separated from it by the character of its elytral maculation. The elytra of *glabrum* are entirely black without any light maculations except occasionally on the humeri and apices. The elytral integument of *teukton* is marked with reddish or light brown maculations which correspond to the pattern of light pubescence. Another external difference is found in the shape of the antennal fossa of the male. *Teukton* shows a shallowly excavated fossa in which the anterolateral wall is very little concave. In *glabrum* the anterolateral wall of the male antennal fossa is more definitely concave and the fossa is moderately deeply excavated.

In a personal communication Maciej Mroczkowski expressed his feeling that this form should be considered nothing more than a variety of *versicolor* (Creutzer). Although resembling *versicolor* in a general way, it is unquestionably distinct. The elytral pattern is without longitudinal lines between the basal loop and the subapical band. The antennal cavity of the male bears fine striations on at least half of its surfaces rather than being microgranulate throughout. The structure of the male genitalia furnishes additional confirmation of the specific separation of the two forms. The bridge of the phallobase in *teukton* is even, narrow, and slightly arcuate. In *versicolor* the bridge is transverse and relatively broad with a thickening at the middle.

T. parabile can most readily be distinguished from *teukton* by its preponderance of golden brown hairs on the pronotum. In *teukton* at least half and often the great preponderance of light colored hairs are white. There are also marked differences in the structure of the male antennae of the two species. *Inclusum* is easily separated from *teukton* by its distinctly emarginate eyes and by the presence of some light longitudinal lines in the elytral maculation.

The species has been found by H. E. Milliron in St. Paul, Minnesota, in a display of silk. Dr. Milliron has also found the species as a pest in whole wheat shipped from North Dakota. Other than this nothing is known of the biology or of the economic potentialities of the species.

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EXPERIMENTAL CROSSING OF MOUNTAIN PINE BEETLE WITH BLACK HILLS BEETLE[1]

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There has long been a controversy as to whether *Dendroctonus monticolae* Hopk. and *D. ponderosae* Hokp., should be considered as one or as two species. The two bark beetles were originally described by A. D. Hopkins from widely separated areas. Hopkins (1902) named and briefly described *D. ponderosae*, and later, in referring to *D. monticolae* (Hopkins 1905) he indicated how the two species may be distinguished morphologically. Hopkins (1909) gave detailed descriptions of the adults and immature forms of both species.

These two great populations of *Dendroctonus* beetles, each of which has killed millions of forest trees, are now known to be in contact. The two species are so similar in morphological characters, habits, and life history that they cannot be distinguished with certainty. The present method of separation is by geographical location (fig. 1), hosts, and size of adults. Blackman (1938), presenting findings on examinations of *D. ponderosae* and *D. monticolae*, noted that it was difficult to distinguish the two species in certain infestations where distributions overlapped. The host tree offers little or no aid in identification, since either species of bark beetle may attack any of several species of pine found in the area. Blackman examined 2888 specimens from localities having typical *D. ponderosae*, such as the Black Hills and Uncompahgre National Forests of South Dakota and Colorado, typical *D. monticolae*, from the Beaverhead and Coeur d'Alene National Forests of Montana and Idaho, and intermingling specimens, from the Medicine Bow and Ashley National Forests of Wyoming and Utah. The typical *D. monticolae* was distinguished from the typical *D. ponderosae* as having an average smaller size and prevailing moderately impressed elytral striae, with distinct but moderately coarse punctures. Blackman found that many specimens do not show average conditions either as regard size or sculpture. Thus, in any series of specimens of either species, individuals will be found which show more or less strongly the characters of the other species. It is Blackman's opinion that, since specimens which intergrade are found so widely, in all probability, one is dealing with a single species which varies according to host, condition of food supply, and region.

Systematics is developing to the point where morphological differences or likenesses are no longer the only criterion for species identification. Huxley (1940) believes that a number of criteria must be taken into account—morphological difference, failure to interbreed, infertility of offspring; ecological, geographical, or genetical distinctness. None of them is singly decisive, however. Failure to interbreed or to produce fertile offspring is the nearest approach to a positive criterion. This latter criteria is the one that was tested in this experiment. The

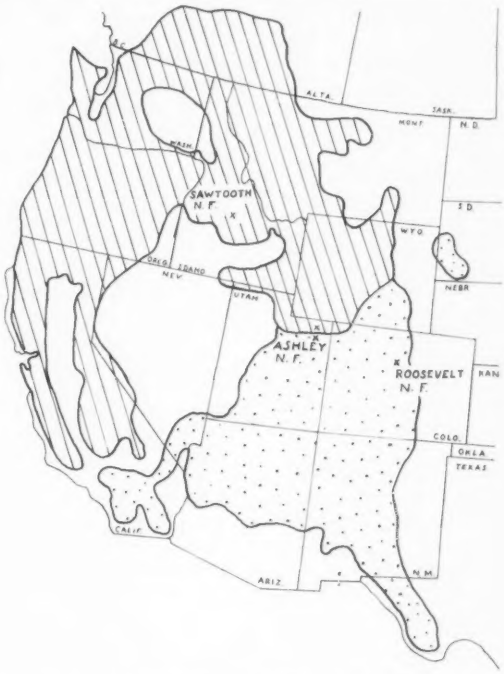

FIG. 1. Distribution of Black Hills beetle and mountain pine beetle.

LEGEND

[::] = Black Hills Beetle

[//] = Mountain Pine Beetle

× = Location where population used for this study was obtained.

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two species were crossed to determine the fertility of the F_1 generation and to study size variations of the beetles resulting from the crossing.

MATERIAL AND METHODS

In this study, crosses were made between the two populations located in the northern and southern localities of the distributional range and between the two intermingling populations. *D. monticolae* infested logs were obtained from lodgepole pine trees on the Sawtooth National Forest of central Idaho and on the Ashley National Forest of northern Utah. *D. ponderosae* infested logs were obtained from ponderosa pine trees on the Roosevelt National Forest of northcentral Colorado and on the Ashley National Forest (fig. 1). The crosses were made only in green ponderosa pine logs, 1½ feet in length and 6 to 8 inches in diameter, obtained from the Roosevelt National forest.

The rearing and crossing program required one year to complete, from September, 1950, to September, 1951, and was carried out in two basement rooms where the humidity and temperature conditions were fairly constant and favorable to beetle development. In the earthern basement the relative humidity varied from 38 to 48 percent and averaged 44 percent, while the air temperature varied from 77° F. to 80° F. and averaged 78° F. In the concrete basement the humidity varied from 30 to 38 percent, averaging 34 percent; and the temperature varied from 62° F. to 69.5° F., averaging 65° F. The concrete room proved the more favorable as mold contamination in the logs was considerably less than that in the earthern room.

The original populations were crossed, giving the F_1 generations, which were selfed to give the F_2 generations. Along with each generation, check populations involving both parental species were reared (Table I). To insure against uncontrolled mating, beetles used in the crossing experiments were removed from the host log while still in the immature stage and reared to adults individually in vials. Even though extreme care was taken in removal of the bark, some mechanical injury was certain to occur. A hand axe and a hammer were the only tools used in cutting and prying the bark loose. The vials used were 1⅜″ by 5/16″. Phloem strips, cut from a green ponderosa pine log, were soaked in a solution of sodium propionate (1 tablespoon to 1 cup water) and allowed to dry 3 to 4 minutes before being placed in the vials. This treatment is thought to minimize mold development. A hollowed-out space was made in the top of the strip, into which the mature larva, pupa, or callow adult was carefully placed. The callow adults were not more than a few days removed from

TABLE I

SUCCESSFUL ATTACKS AND DEGREE OF FERTILITY FOR THE MATED POPULATIONS OF THE MOUNTAIN PINE BEETLE AND THE BLACK HILLS BEETLE

Populations Mated	No. of Logs	No. of Matings	No. of Successful Attacks[a]	% of Successful Attacks	Inches of Adult Galleries	No. of Larval Tunnels	Ave. Gallery Length Per Attack	Ave. No. of Larval Tunnels Per Attack
1. *D. monticolae* (Lp)[b], S. N. F.) X *D. ponderosae* ((P), R. N. F.)	7	34	16	47	286	988	18	62
2. *D. monticolae* (Lp. A. N. F.) X *D. ponderosae* (P, A. N. F.)	5	22	13	59	250	911	19	70
3. *D. monticolae* (Lp, S. N. F.) X *D. monticolae* (Lp, S. N. F.) check	5	25	21	84	427	1731	20	82
4. *D. ponderosae* (P, R. N. F.) X *D. ponderosae* (P, R. N. F.) check	4	18	12	67	230	1041	19	87
5. Selfing of F_1 from No. 1 cross above	10	51	27	53	552	2310	20	86
6. Selfing of F_1 from No. 2 cross above	4	17	2	12	36	222	18	111
7. Selfing of F_1 from No. 3 check aboye	5	21	8	38	126	587	16	73
8. Selfing of F_1 from No. 4 check above	4	19	4	21	142	416	36	104

[a]Not included are 17 attacks where an adult gallery was present, but larval tunnels were absent.
[b]Lp, lodgepole pine; Pp, ponderosa pine; S. N. F., Sawtooth National Forest; R. N. F., Roosevelt National Forest; A. N. F., Ashley National Forest.

the pupal stage when taken from the logs. Their external covering was exceedingly pale in color and was still soft and tender. They had not yet started to enlarge their pupal chambers by feeding on the surrounding walls. The vials were stoppered firmly with absorbent cloth, and then placed in sealed coffee cans. The old phloem strips were replaced by fresh ones every one or two weeks. When the callow adults blackened at maturity, the sex of the beetles was determined and length and width measurements were taken with a micrometer.

The beetles were then paired at random and forced to attack in green ponderosa pine bolts. Close to the base of the log, a hole, 1/8 inch in diameter, was drilled through the bark. This served as an entrance hole for the adult beetles. The female was introduced into the hole first, followed by the male. A one-inch square piece of screen wire was tacked over the hole to force the beetles to make their attack at that point. A minimum of three inches was set for the distance between attacks. When the bolt had its quota of attacks, the base of the bolt was nailed to a square board and immediately caged securely in Cel-O-Glass (14 mesh galvanized screen wire coated on both sides with cellulose acetate) to prevent the escape of any emerging adults. (Refer to Table I for the number of logs used and the number of matings involved for each set of populations mated, cross or check.) The female parent adult mined a vertical, nearly straight gallery from the point of attack at the base of the log. The gallery often covered the full 18 inch length of the log. In a number of cases, when the female neared the top of the log, she would arch either to the right or to the left and continue back down the log. The galleries never crossed, as the females apparently are able to sense when their mine approaches another gallery. The galleries were rarely closer than 1½ inches to each other.

RESULTS AND DISCUSSION

Degree of fertility.—The two populations, that of the mountain pine beetle in Idaho and the Black Hills beetle in Colorado, showed the ability to produce fertile offspring when crossed. Table I shows that the percentage of successful attacks varied from 47 to 84 percent for the crosses between the mountain pine beetle and the Black Hills beetle and their checks. A successful attack was one in which an adult gallery and larval tunnels had been mined, indicating that fertile eggs resulted from the mating. If no larval tunnels were formed, which was the case with some matings, it could not be determined whether this was due to infertile eggs or to early death of larvae.

The cross between *D. ponderosae* and *D. monticolae* from the Ashley National Forest resulted in only two fertile matings, or 12 percent successful attacks when the F_1 was selfed. The checks from the selfing of the F_1 checks show a decided decrease in percentage of successful attacks, especially for the *D. ponderosae* check which decreased from 67 to 21 percent.

Table I shows that the "average gallery length per attack" varied from 16 to 36 inches, and the "average number of larval tunnels per attack" varied from 62 to 111. The overall average per attack was a 20 inch gallery containing 80 larval tunnels. Research by Blackman (1931) on the Black Hills beetle revealed that under field conditions, an average adult gallery of 22 inches would contain about 184 hatched larvae. The average number of eggs which hatched per attack, measured in terms of larval tunnels, varied from 62 to 89 for the cross between *D. monticolae* of the Sawtooth National Forest and *D. ponderosae* of the Roosevelt National Forest. The fertility in the cross between *D. monticolae* of the Ashley National Forest and *D. ponderosae* of that same forest was found to increase from 70 larval tunnels to 111 tunnels. In this case, the 111 tunnels are based on only two successful attacks.

The sex ratio favored the females slightly in all but the F_2 generations, where the ratio was 1.3 males to 1 female.

Analysis of size variations.—The length and width measurements of the adult beetles in the various populations were analyzed by use of a variance and covariance study. There were three groups of beetles (Table II): group I consisted of the four original parent populations; group II, the two F_1 generations and checks; and group III, the two F_2 generations and two checks. Each population within its group was divided according to the sex.

The beetles reared artificially in groups II and III were significantly smaller in length than the beetles which developed under more nearly natural conditions in group I. The means of each group showed a gradual decrease with each group from 5.38 mm. for group I, to 5.28 mm. for group II, and 5.26 mm. for group III. In addition, the F_1 and F_2 checks from the Roosevelt parents showed decreases in their means. The decrease in means may be due to rearing the beetles under laboratory conditions which could not duplicate entirely the natural conditions. Growth may have been stunted when some of the beetles were removed from the host log while still immature and forced to feed in vials. The F_1 and F_2 checks from the Sawtooth parents reacted differently. There was a decrease in size in the F_1, but then an increase occurred in the F_2. This deviation might be explained in part by findings of Blackman (1938) and De Leon (1939). Blackman noted that Black Hills beetles from hosts other than ponderosa pine, especially from lodgepole pine, were often smaller in average size. De

Leon found that in a rearing study using the Black Hills beetle, the mean size of the adult beetles was less in lodgepole pine than in ponderosa pine. It would follow that mountain pine beetles taken from lodgepole pine and reared in ponderosa pine could be expected to increase in size.

In group I the beetles of the parental population taken from their natural host, ponderosa pine, were found to be significantly larger than those of the parental population taken from lodgepole pine. The females were significantly larger than the males in all cases. Highly significant positive correlation between length and width of beetles was found in all but one instance.

The results from the crossing of two heterogenous populations, which differ in a size characindividual beetles in a population about the mean length.

The Sawtooth x Roosevelt beetles in this experiment bear out the second possibility fairly well, for when beetle lengths and widths were analyzed for the various populations, it was found that in the F_1 and F_2 generations the mean was significantly different from and intermediate between both parents; and that variability in the F_2 was similar to both parents instead of exceeding that of either parent population. However, the class range (shown in Table II) is greater than that of either parent.

The data from the Ashley Lp x Ashley PP beetles, did not fit the second hypothesis as well as in the instance of the Sawtooth x Roosevelt cross. In the F_1 and F_2 generations, the means were smaller than the parents, instead of inter-

TABLE II

RANGE, MEAN, STANDARD DEVIATION, AND COEFFICIENT OF VARIABILITY OF THE VARIOUS POPULATIONS FOR LENGTH OF ADULT BEETLES, MEASURED IN MILLIMETERS

	Population	N	Range	Mean	S.D.	Coefficient of Variability %
Gr. I	*D. monticolae* Parents-S. N. F. (Lp)	200	3.7–6.5	5.09± .04	0.54± .03	10.61± .54
	D. ponderosae Parents-R. N. F. (Pp)	141	4.7–6.9	5.64± .04	0.52± .03	9.22± .55
	D. monticolae Parents-A. N. F. (Lp)	142	3.8–6.6	5.39± .04	0.51± .03	9.46± .57
	D. ponderosae Parents-A. N. F. (Pp)	128	4.0–6.5	5.52± .05	0.53± .03	9.60± .60
Gr. II	*D. monticolae*-S. N. F. (Lp) X *D. ponderosae*-R. N. F. (Pp)F_1	342	4.0–6.5	5.34± .03	0.50± .02	9.36± .36
	D. monticolae-A. N. F. (Lp) X *D. ponderosae*-A. N. F. (Pp)$_1$	139	3.7–6.0	4.96± .04	0.47± .03	9.48± .57
	D. monticolae X *D. monticolae* S. N. F. (Lp)F_1 check	237	3.7–6.0	4.99± .03	0.43± .02	8.62± .40
	D. ponderosae X *D. ponderosae* R. N. F. (Pp)F_1 check	256	4.5–6.8	5.63± .03	0.49± .02	8.70± .39
Gr. III	*D. monticolae*-S. N. F. (Lp) X *D. ponderosae*-R. N. F. (Pp)F_2	568	4.0–7.0	5.25± .02	0.51± .01	9.71± .29
	D. monticolae-A. N. F. (Lp) X *D. ponderosae*-A. N. F. (Pp)F_2	42	4.7–5.8	5.18± .05	0.31± .03	5.98± .65
	D. monticolae X *D. monticolae* S. N. F. (Lp)F_2 check	143	3.9–6.4	5.16± .04	0.48± .03	9.30± .55
	D. ponderosae X *D. ponderosae* R. N. F. (Pp)F_2 check	155	4.3–6.6	5.39± .03	0.42± .03	7.79± .44

ter (such as discussed here) should conform with one of two possibilities. (1) If the two parents belong to different species, the size of the F_1 approaches the larger parent or exceeds it, because of heterosis, and the F_2 does not result, as the F_1 is usually sterile. (2) If both parents are one and the same species, the mean size of the F_1 generation is intermediate between the parents and variability is similar to that of the parents; in the F_2 generation, the mean size is intermediate between those of the two parent populations and about the same as the F_1 generation; variability increases considerably and exceeds that of either parent population.

The variability existing between the various populations is expressed as a percentage when reference is made to the coefficient of variability in Table II. Variability as used here may be defined as a measure of the dispersal of the mediate between them. Since the parents were only slightly different in size, and since the F_1 and F_2 were artificially reared, it is to be expected that the beetles would be smaller.

The percentage of fertile matings resulting from the selfing of F_1 progeny of the Sawtooth x Roosevelt cross exceeded the percentage for the corresponding check populations. This fairly high percentage of successful attacks (53) and the close agreement of the quantitative data on size variations with the hypothesis of both species (*D. ponderosae* and *D. monticolae*) acting as a true single species gives evidence in favor of actually considering the two species as one. There is a need for a cytological investigation to determine if there is any difference in the chromosomal configuration of the two species, thus adding or detracting from the presented evidence supporting the single species hypothesis.

SUMMARY

The hypothesis tested in this thesis concerns the relationship between two described species of *Dendroctonus:* the mountain pine beetle, *D. monticolae*, and the Black Hills beetle, *D. ponderosae*. The two species are so similar in morphological characters, habits, and life history that they cannot be distinguished with certainty. Experiments were based on the fact that failure to interbreed or to produce fertile offspring is the nearest approach to a positive criterion for species identification.

Crosses were made between the two populations located in the northern and southern localities of the distributional range and between the two intermingling populations. *D. monticolae* infested logs were obtained from lodgepole pine trees in the Sawtooth National Forest of central Idaho and in the Ashley National Forest of northern Utah. *D. ponderosae* infested logs were obtained from ponderosa pine trees in the Roosevelt National Forest of north-central Colorado and in Ashley National Forest.

Fertile offspring resulted when the mountain pine beetle in Idaho was crossed with the Black Hills beetle in Colorado. The original matings showed 47 percent successful attacks and the selfing of the F_1 progeny, 53 percent.

An analysis of the length and width measurements for the various populations involved in the cross showed that the mean for the F_1 and F_2 generations was significantly different from and intermediate between both parents and that the variability in the F_1 and F_2 was similar to both parents.

The above facts favor the consideration that the two nominal species be classified as one.

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PAPER CHROMATOGRAPHY OF FRESH TISSUE EXTRACTS OF SUSCEPTIBLE AND INSECTICIDE-RESISTANT STRAINS OF THE HOUSE FLY, MUSCA DOMESTICA L.[1]

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Possible differences between susceptible and insecticide-resistant strains of the house fly, *Musca domestica* L., other than those related to responses to a chemical treatment, have occupied a variety of research efforts. Among these have been attempts to correlate resistance with morphological changes. Wiesmann (1947) reported a number of structural differences between a resistant strain and a susceptible strain and suggested that these differences might account for the variance in susceptibility. Other workers have found no differences, or only small variations in structure, which could not account for the great differences in tolerance to insecticides (D'Alessandro *et al.* 1949, Anonymous 1950, and March and Lewallen 1950). Bigelow and LeRoux (1954) reported statistically significant morphological differences between a number of susceptible and DDT-resistant strains which could be used as diagnostic characteristics in separating the susceptible and resistant strains.

Numerous workers have reported differences between susceptible and resistant strains in egg production and viability, in length of life cycle, body weight, oxygen consumption, and activities of various enzymes. Varzandeh *et al.* (1954) have shown that similar variations may occur in susceptible strains as well as in resistant strains, and they have pointed out that such differences

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may explain the conflicting conclusions regarding the effect of the acquisition of resistance upon unrelated characteristics.

In a biochemical approach to this problem Sternburg *et al.* (1954) have compared DDT-dehydrochlorinase activity in a number of susceptible and resistant strains. No activity could be detected in four susceptible strains, but in ten resistant strains the DDT-dehydrochlorinase activity was directly correlated with the resistance of the strain. Perry *et al.* (1953) proposed a technique for determining DDT-resistance in flies by measuring the *in vivo* conversion of DDT to DDE.

Recently, a technique has been developed for showing biochemical differences in morphologically identical strains of *Drosophila melanogaster* Meigen (Buzzati-Traverso 1953), in several species of fish (Buzzati-Traverso and Rechnitzer 1953), in the *Culex pipiens* complex (Micks 1954), in several species of land snails (Kirk *et al.* 1954), and in a number of plants and citrus rootstocks (Buzzati-Traverso 1953, Selle 1954) by means of paper chromatography of fresh tissues or body fluids. The present study was undertaken to determine whether or not biochemical differences peculiar to susceptible and resistant strains of the house fly could be discerned by means of this chromatographic technique.

MATERIALS AND METHODS

Rearing of Fly Populations.—For rearing of populations of the house fly, *Musca domestica*, the larval medium for each jar consisted of the following well-mixed ingredients: 12.5 ml. concentrated Diamalt (Standard Brands Incorporated, New York, N.Y.), 600 ml. water, 30 gm. dried Brewer's yeast (Standard Brands Incorporated, New York, N.Y.), 112 gm. micro-mixed Purina Laboratory Chow (Ralston Purina Company, St. Louis, Mo.), and 225 gm. milled red wheat bran. Eggs were collected from cotton oviposition pads saturated with evaporated milk and water (50: 50) in a 9-cm. petri dish. The eggs were washed in distilled water and 0.3 ml. were measured into the rearing medium held in a 1-gallon glass mayonnaise jar. Following larval development and pupation, the adults were permitted to emerge in the rearing jars. Newly emerged adults, not over 24 hours old were collected and used in the experiments.

Populations of the little house fly, *Fannia canicularis* (L.), were reared by the method developed by Lewallen (1954), and those of the greenbottle fly, *Phaenicia sericata* (Meigen), were reared on horse meat, after the method of Miller (1940).

Strains of Flies Used.—The following nine strains of the house fly were used in these studies:

1. Stauffer—a susceptible laboratory strain, originally subsampled from a Berkeley laboratory strain.
2. Riverside—a susceptible laboratory strain, originally subsampled from a Berkeley laboratory strain.
3. Orlando—a susceptible laboratory strain.
4. Savannah—a susceptible laboratory strain.
5. DDT-resistant—developed from the Riverside strain by selection with DDT.
6. Prolan-resistant—developed from the Riverside strain by selection with Prolan (bis-(*p*-chlorophenyl) 2-nitropropane).
7. DDT-synergist-resistant—developed from a multiple-resistant field-collected strain by selection with DDT plus bis-(*p*-chlorophenyl) chloromethane (March *et al.* 1952).
8. Bellflower—field collected in 1948 (March and Metcalf 1949a) and at that time highly resistant to DDT and related insecticides; subsequently reared in the laboratory without exposure to insecticides and at present exhibiting only a low order of resistance.
9. Super-Pollard—field collected in 1949 (March and Metcalf 1949b); resistant to DDT, lindane, dieldrin, and related insecticides; subsequently reared in the laboratory under continuous selection with DDT and lindane.

Strains of the little house fly and the greenbottle fly were field collected and subsequently reared in the laboratory.

Preparation of Fly Extracts for Chromatography.—Homogenates of adult flies, larvae, or pupae were prepared by grinding 100 whole specimens in 2 ml. of distilled water in an all-glass Potter-Elvehjem homogenizer. The homogenates were centrifuged at 1000 times gravity for 5 minutes, and the supernatants were poured off and pipetted along a line 2 cm. from the bottom and 2.5 cm. from the sides of squares (22×22 cm.) of Whatman No. 1 filter paper. The lines thus formed averaged about 5 mm. in width and 170 mm. in length. All papers were prepared in triplicate.

Chromatography.—After preparation, the papers were dried for 24 hours and then formed into cylinders by stapling the sides together so that the edges did not touch. Chromatography after the method of Buzzati-Traverso (1953) was accomplished by the ascending technique, by placing the paper cylinders in 9-cm. petri dishes containing 20 ml. of the mobile solvent inside of a cylindrical battery jar (30 x 30 cm.) covered with a glass plate. The vapor phase of the solvent was allowed to reach equilibrium at 18 degrees C. before introduction of the paper cylinder. The mobile solvent, which consisted of two parts of *n*-propanol and one part 1 percent aqueous ammonia, was allowed to rise to within 2 cm. of the top of the paper cylinder, usually requiring about a 7- to 8-hour run. At this time the papers were removed and dried in the air at room temperature. The chromatograms were examined under ultraviolet light filtered to produce

radiations in the region of 3600 Å, or sprayed with 0.2 percent ninhydrin in 95 percent ethanol, with 5 percent 2,4,6-collidine added just before using. The ninhydrin-positive pattern developed in 1 to 2 hours at room temperature. The developed chromatograms were examined principally for qualitative differences, no extended attempt being made to determine quantitative differences or to identify the chemical nature of the various components producing the patterns.

EXPERIMENTAL RESULTS

In preliminary trials, fluorescent patterns were compared with ninhydrin-positive patterns. Since in all cases the ninhydrin-positive patterns showed the greater number of lines and the more easily distinguished patterns, they were used for most of the comparisons. Figure 1 illustrates the ninhydrin-positive patterns for the Stauffer and DDT-synergist-resistant strains.

The effect of sex and eye pigment on the chromatographic patterns was examined in the Stauffer strain. Homogenates of whole male flies gave the same pattern as whole female flies. Decapitated females produced the same pattern as whole females, and the heads alone produced the same pattern as decapitated or whole females. Chromatographic patterns of homogenates of larvae and pupae as well as of adults of several strains also were examined, but no differences were evident.

Comparisons of four susceptible strains of house flies and five insecticide-resistant strains in terms of R_f values for the chromatographically separated ninhydrin-positive components of homogenates of adult females are given in Table I. The susceptible Stauffer strain had a line with an R_f of 0.08 that was not found in any of the other house-fly strains. The susceptible Orlando and Savannah strains had lines with R_f's of 0.86 and 0.96 and 0.88 and 0.97 respectively, which were not found in any of the other strains; in addition, the Savannah strain was unique in having a line at R_f 0.46. The Savannah strain had the greatest number of lines—11 in all. The R_f values of the lines from the susceptible Riverside strain and all the resistent strains were the same, seven well-defined lines being evident for each. In the Bellflower strain, the line at R_f 0.13 was 6 mm. wide, while in all the other strains this line was 3 mm. wide.

R_f values for the chromatographically separated ultraviolet-fluorescent components of homogenates of adult females of four susceptible strains and four resistant strains are given in Table II. The fluorescent patterns agree quite well with the ninhydrin patterns, although some of the more distinguishing lines of the ninhydrin patterns did not fluoresce. It is of interest that the unique ninhydrin-positive lines at R_f 0.08 for the Stauffer strain and at R_f 0.46 for the Savannah strain appeared to be present in the

TABLE I

R_f VALUES FOR CHROMATOGRAPHICALLY SEPARATED NINHYDRIN-POSITIVE COMPONENTS OF HOMOGENATES FROM ADULT FEMALE HOUSE FLIES OF VARIOUS STRAINS

STRAIN	R_f VALUES OF NINHYDRIN-POSITIVE LINES											
Stauffer	0.08	0.13	0.20	0.26	0.31	0.37	—	0.54	0.64	0.72	—	—
Riverside	—	0.13	0.20	0.27	—	0.39	—	0.55	0.65	0.72	—	—
Orlando	—	0.13	0.20	0.27	0.34	0.40	—	0.54	0.62	0.74	0.86	0.96
Savannah	—	0.14	0.23	0.29	0.34	0.40	0.46	0.57	0.69	0.77	0.88	0.97
DDT-resistant	—	0.13	0.20	0.27	—	0.39	—	0.55	0.65	0.72	—	—
Prolan-resistant	—	0.13	0.20	0.27	—	0.39	—	0.55	0.65	0.72	—	—
DDT-synergist-resistant	—	0.13	0.20	0.27	—	0.39	—	0.55	0.65	0.72	—	—
Bellflower	—	0.13[a]	0.20	0.27	—	0.30	—	0.55	0.65	0.72	—	—
Super-Pollard	—	0.13	0.20	0.27	—	0.39	—	0.55	0.65	0.72	—	—

[a]Line 6 mm wide; all other lines at this R_f 3 mm. wide.

TABLE II

R_f VALUES FOR CHROMATOGRAPHICALLY SEPARATED ULTRAVIOLET-FLUORESCENT COMPONENTS OF HOMOGENATES FROM ADULT FEMALE HOUSE FLIES OF VARIOUS STRAINS

STRAINS	R_f VALUES OF ULTRAVIOLET FLUORESCENT LINES									
Stauffer	0.07	—	—	0.27	—	0.36	0.46	0.58	—	0.98
Riverside	0.11	0.14	0.19	—	0.31	0.38	0.47	0.60	—	0.98
Orlando	0.10	0.14	—	0.25	0.33	0.41	0.50	—	0.69	0.98
Savannah	0.11	0.15	—	0.24	0.32	0.40	0.50	—	0.67	0.98
DDT-resistant	0.08	0.12	0.20	—	0.28	0.35	0.45	0.60	—	0.98
Prolan-resistant	0.08	0.14	0.23	—	0.31	0.40	0.49	0.61	—	0.98
DDT-synergist-resistant	0.12	—	0.18	0.26	—	0.38	0.46	0.54	0.69	0.98
Super-Pollard	0.09	0.16	—	0.25	0.34	—	0.46	0.59	—	0.98

fluorescent patterns of all the strains. Apparently, a quantitative difference in the amount of material in the various strains is involved here. Similar quantitative considerations may hold for some of the other lines which differ in the two sets of patterns.

When the susceptible Stauffer male was crossed with the DDT-synergist-resistant female, the chromatographic ninhydrin-positive pattern of the F_1 female was the same as that for the susceptible Stauffer male parent, *i. e.*, line at R_f 0.08 present. In the reciprocal cross of DDT-synergist-resistant male with Stauffer female, the pattern of the F_1 female was the same as for the DDT-synergist-resistant male, *i. e.*, line at R_f 0.08 absent. Thus in each cross, the F_1 female showed the same pattern as the male parent.

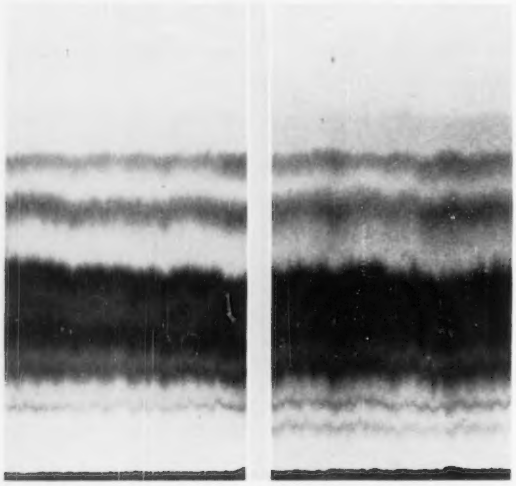

FIG. 1. Pattern for chromatographically separated ninhydrin-positive components of homogenates from adult female house flies; (DDT-synergist-resistant strain, chromatogram at left; Stauffer strain, chromatogram at right).

For comparative purposes the ninhydrin-positive chromatographic patterns of the green-bottle fly and the little house fly, which are frequently found inhabiting the same sites as the house fly, were examined (See Table III). The patterns of these two species show distinct differences, and they likewise differ from the house-fly patterns, although they contain many of the same lines as the house-fly patterns.

DISCUSSION OF RESULTS

Although differences in the chromatographic patterns of the susceptible and insecticide-resistant house-fly strains were readily demonstrated, none of these differences could be directly correlated with either susceptibility or resistance. Thus the principal objective of this study, to find biochemical characteristics peculiar to resistant house flies by means of chromatographic techniques, was not accomplished. A number of interesting secondary results were obtained, however.

The differences between strains appeared to be correlated with the origins of the strains. The Stauffer and Riverside strains and Orlando and Savannah strains were obtained from widely separated geographical areas. The chromatographic patterns of the two strains from California more closely resembled each other then those of the two strains from the southeast section of the country, which were more similar to each other than to the California strains. All four strains had distinct and unique chromatographic patterns, however. The five resistant strains were either derived by insecticidal selection from the Riverside strain or were field collected in California, as was the Riverside strain originally. All five resistant strains showed the same ninhydrin-positive chromatographic pattern as the Riverside strain. Thus, resistant strains developed in the laboratory from this strain showed no changes in chromatographic pattern, and field-collected strains from the same general area likewise showed no changes. The ultraviolet-fluorescent patterns showed differences between the Riverside strain and two of the three field-collected resistant strains but no differences between the Riverside strain and the two resistant strains derived from it in the laboratory.

TABLE III

R_f VALUES FOR CHROMATOGRAPHICALLY SEPARATED NINHYDRIN-POSITIVE COMPONENTS OF HOMOGENATES FROM ADULTS OF *Phaenicia sericata* AND *Fannia canicularis*.

LINE	R_f VALUES OF NINHYDRIN-POSITIVE LINES	
	P. sericata	*F. canicularis*
1	....	0.08
2	0.15	0.12
3	0.24	0.21
4	0.29	0.29
5	0.40	0.38
6	0.57	0.50
7	0.75	0.70
8	....	0.87

This study lends support to the conclusions of Varzandeh *et al.* (1954) concerning the heterogeneity of various house-fly strains and the independent nature of various characteristics unrelated to resistance. It also suggests an explanation for the results of Bigelow and LeRoux (1954), who reported statistically significant morphological differences between a number of non-DDT-resistant strains (California, Mayo, Methoxychlor, and Macdonald) and DDT-resistant strains (Bellflower, Pollard, and Beltsville). The Methoxychlor strain, which was

developed by selection with methoxychlor from the Riverside strain (designated California strain by Bigelow and LeRoux), should have been classified with the DDT-resistant group, since a high level of resistance to DDT developed concurrently with resistance to methoxychlor. This fact was unknown to Bigelow and LeRoux. Thus, their characters are not uniquely related to DDT-resistance but are independent in nature, and the close morphological relationship of the Riverside and Methoxychlor strains, since the latter was developed from the former, might have been expected.

The marked differences in chromatographic patterns of the three species, *Musca domestica*, *Fannia canicularis*, and *Phaenicia sericata*, further suggest the possible usefulness of this technique in taxonomic studies, as previously suggested (Buzzati-Traverso 1953, Buzzati-Traverso and Richnitzer 1953, Micks 1954, and Selle 1954). Caution should be exercised in interpreting results, however, since four chromatographic patterns which are as distinct as those separating the three species were found for the nine house-fly strains.

SUMMARY AND CONCLUSIONS

Four susceptible and five insecticide-resistant strains of the house fly, *Musca domestica* L., and strains of *Fannia canicularis* (L.) and *Phaenicia sericata* (Meigen) were examined by means of paper chromatography of fresh tissue extracts. A number of differences in the chromatographic patterns of the nine house-fly strains were found, but none of these differences could be directly correlated with susceptibility or resistance. Instead, they seemed to be correlated with the origin of the strain.

This study supports other work on the heterogeneity of both susceptible and resistant house-fly strains and the independent nature of various characteristics unrelated to resistance.

In addition it suggests that caution must be exercised in interpreting results of taxonomic studies with chromatographic techniques since chromatographic patterns which are as distinct as those separating three different species, *Musca domestica*, *Fannia canicularis*, and *Phaenicia sericata*, were found among the nine house-fly strains examined.

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PAPER CHROMATOGRAPHY IN INSECT TAXONOMY[1]

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INTRODUCTION

Although chromatography is a comparatively old procedure, the method of paper partition chromatography did not become popular until well after 1944 when Consden, Gordon and Martin introduced it and demonstrated its application. This tool was given renewed interest and greater utility as a result of the development of the ascending method of paper chromatography by Williams and Kirby in 1948. Since that time, chromatographic procedures with paper have been applied by a host of investigators to natural, synthetic, organic and inorganic substances. Bacteriologists, geneticists, botanists, zoologists and a variety of others have employed paper chromatography as an analytical method for the study of proteins, amino acids, carbohydrates, steroids, antibiotics, vitamins and many other substances.

Entomologists and others interested in insect biochemistry and nutrition have employed paper partition chromatography mainly for the purpose of studying the amino acids of insects. For example, the hemolymph of various immature and adult insects has been analyzed by this method for its amino acid composition by Raper and Shaw (1948), Finlayson and Hamer (1949), Agrell (1949), Levenbook (1950), Pratt (1950) and others. Certain other specific components such as chromosomes and salivary gland secretions of insects have been subjected to paper chromatographic procedures by Blumel and Kirby (1948), Kodani (1948), Yasuzumi and Miyao (1950) and Moorefield and Fraenkel (1954).

Aside from the use of paper chromatography as an analytical tool, its application to taxonomy was not suggested until relatively recent years. Following chromatographic studies of mosquito extracts which were begun in our laboratory in 1949, it became apparent that paper chromatography might have some merit as a taxonomic method (Micks and Ellis, 1951). Subsequent investigations by Hadorn and Mitchell (1951), Micks and Ellis (1952), Ball (1952, 1953), Buzzati-Traverso (1953), Hadorn and Kühn (1953) and Micks (1954) have provided further evidence that chromatography can be successfully applied to problems in systematics.

It is the principal purpose of this report to point out the various chromatographic techniques which may be employed by entomologists to assist them in solving taxonomic problems. Results obtained by us in utilizing these methods are presented herein.

METHODS

No attempt will be made to present a detailed review of paper chromatographic methods used by various authors since our own incorporate most of those which have been commonly employed. Throughout our own studies, we have applied paper partition chromatography (Consden, Gordon and Martin, 1944), using the ascending modification of Williams and Kirby (1948). We have made chromatograms of whole, deproteinized mosquitoes as well as mosquito extracts and have compared both the free amino acid content and the patterns of fluorescent substances.

Our first work was done with extracts prepared in the following manner. Unfed, adult female mosquitoes were collected 24 to 48 hours after emergence and lightly anesthetized with chloroform. They were immediately weighed and homogenized in a tissue grinder with 1 ml. of distilled water. The material was centrifuged at 2,000 RPM and the supernate retained. Protein precipitation was carried out by adding one volume of 95% ethanol to approximately 5 ml. of sample and centrifuging. The supernate was shaken with 3 volumes of chloroform (15 ml.) with subsequent centrifugation at a low speed and the aqueous fraction was removed for amino acid determinations. The mosquito extracts were applied to the paper with a 0.2 ml. pipette. The amount applied to a single spot represented 12 mg. (approximately 6 mosquitoes) for the one-dimensional chromatograms and 40 mg. for the two-dimensional runs.

Our other method of preparation is a modification of the techniques of Hadorn and Mitchell (1951) and Buzzati-Traverso (1953). Whole mosquitoes were boiled for two minutes in distilled water to coagulate the protein. The excess water was removed by touching the specimens to a piece of blotting paper and the bodies were mashed on the chromatographic paper with a glass rod with a flattened end. Four adult female mosquitoes were used to make a single preparation and each species was always run in duplicate. The spot produced by each specimen was allowed to dry before adding the next in order to keep the material confined to a

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small area. A hair dryer served as a source of hot air.

Both one-dimensional and two-dimensional chromatograms of whole mosquitoes and extracts were prepared with Whatman No. 1 filter paper cut into 9″ x 11″ sheets. Scotch tape was used to fasten the chromatograms into cylinders. Propanol and 1% NH_3 (2:1) and propanol-diethylamine-water (85:4:15) were used as one-dimensional solvents. Butanol-acetic acid-water (4:1:1) was employed as the second solvent for two-dimensional chromatograms. All sheets were allowed to dry at room temperature and were sprayed with 0.2% ninhydrin in water-saturated butanol using a nasal atomizer. Many of the chromatograms were examined under ultraviolet light and the fluorescent spots marked prior to ninhydrin treatment.

For certain purposes, it was desirable to make quantitative determinations of the free amino acids present on two-dimensional chromatograms. The amino-nitrogen method of Harding and MacLean (1916) was employed. In preparation for this procedure, a two-dimensional chromatogram, after having been run, was treated with 0.05% ninhydrin in water-saturated butanol and heated at about 80°C. for 5 to 10 minutes. The colored amino acid spots appearing thereon were eluted and compared with known solutions in the spectrophotometer.

Aside from amino acid determinations of insects and their various components, chromatographic procedures may also be applied to certain vitamins. In this case the method usually varies with the vitamin to be analyzed. Extracts of *Anopheles quadrimaculatus* Say, *Culex fatigans* Wied. and *Aedes aegypti* (L.) were analyzed for vitamin A using the method of Datta (1949) and for riboflavin following special chromatographic procedures patterned after Crammar (1948) and Hais (1949).

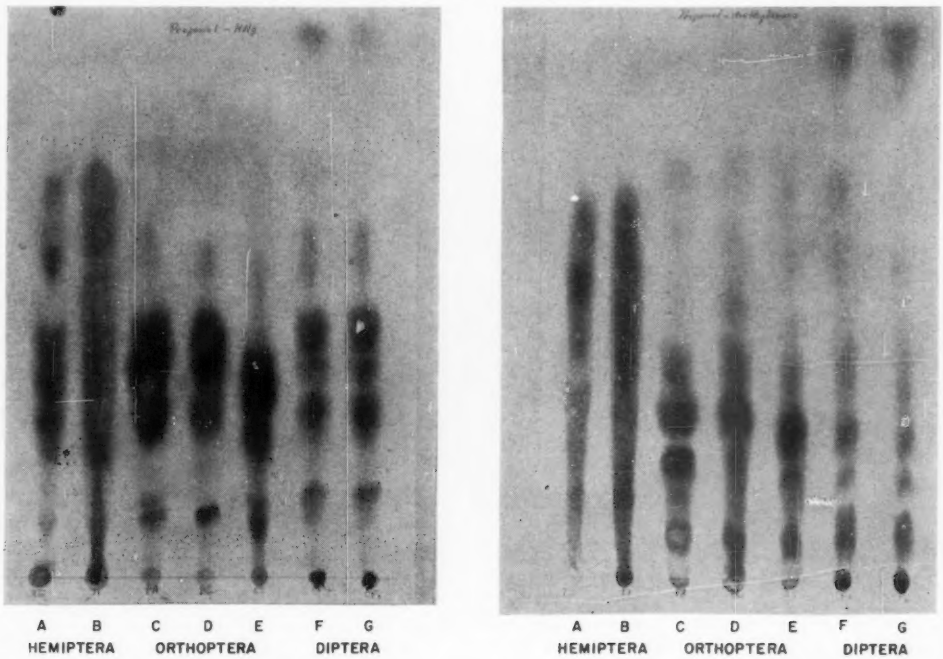

FIG. 1. One-dimensional chromatograms of Hemiptera (*T. gerstaeckeri*, A; *T. infestans*, B), Orthoptera (*Periplaneta americana*, C; *Blattella germanica*, D; *Supella supellectilium*, E) and Diptera (*C. fatigans*, F and G). The chromatogram on the left was run in propanol-NH_3 and the one on the right in propanol-diethylamine.

More recently, we have begun a thorough investigation of quantitative paper chromatography as a taxonomic aid. This procedure entails subjecting developed chromatographic strips to more precise measurement in a densitometer. A characteristic density curve is thereby obtained for a species. It is felt that such a precise method as this may greatly extend the usefulness of paper chromatography and increase the accuracy of the interpretation of the chromatographic findings.

RESULTS

Figure 1 indicates the diversity of amino acid patterns obtained from different orders of insects,

and how various chromatographic solvents may be employed to obtain different patterns of ninhydrin-positive substances. Body fluids of certain Hemiptera (*Triatoma gerstaeckeri* (Stål) and *T. infestans* (Klug), for example present a chromatographic picture which is entirely different from that of some Orthoptera (*Periplaneta americana* (L.), *Blattella germanica* (L.) and *Supella supellectilium* (Serv.)) or Diptera (*Culex fatigans*). Furthermore, marked differences between the chromatograms of the three genera of cockroaches are obvious. The ninhydrin-positive patterns obtained from the Diptera the latter. This same method has also been successfully used in distinguishing between two different strains of *C. molestus*.

Figure 3 illustrates the patterns of amino acids which were obtained following the application of two-dimensional chromatographic methods to mosquito larvae. A comparison of fourth-stage larvae of *A. quadrimaculatus* and *C. molestus* in this figure demonstrates quantitative differences in several of the amino acids. For example, spots number 1 and 4 are more intense in *C. molestus* larvae, whereas spots number 2 and 3 are darker in *A. quadrimaculatus*. The same

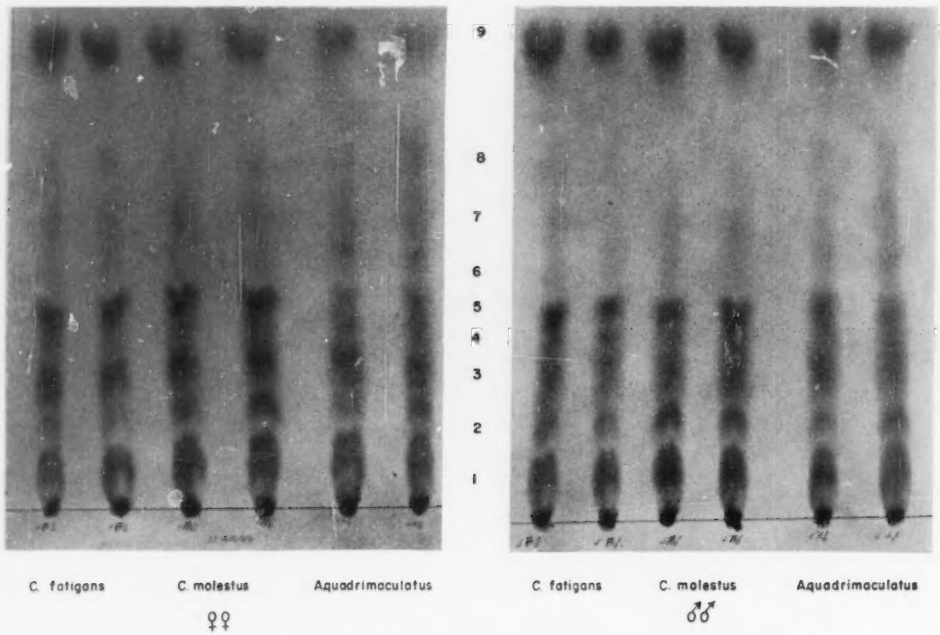

FIG. 2. One-dimensional chromatograms of whole specimens of female and male adults of *C. fatigans*, *C. molestus* and *A. quadrimaculatus* run in duplicate.

represent duplicate preparations of *C. fatigans* showing the reproducibility of the method.

Chromatograms (one-dimensional) of both female and male specimens of three species of mosquitoes (*C. fatigans*, *C. molestus* and *Anopheles quadrimaculatus*) are compared in figure 2. It can be noted that the patterns of the two culicine species, though not quantitatively identical, are somewhat different than those obtained from *A. quadrimaculatus*. This difference is demonstrated in both sexes. For example, spot number 5 is always more intense in the two culicine species thereby differentiating their ninhydrin-positive patterns from that of the *Anopheles*. On the other hand, *C. molestus* may be distinguished from *C. fatigans* by a darker spot number 2 in the former and a more dense spot number 9 in number of larvae (7) was used in preparing each chromatogram.

In order to check and extend our chromatographic findings with three mosquito species (*A. aegypti*, *A. quadrimaculatus* and *C. fatigans*) (Micks and Ellis, 1951), amino acid spots were eluted from several two-dimensional chromatograms of each and the color was compared with that of known amino acids in the spectrophotometer. Table I compares those amino acids on which analyses have been completed. On the basis of an average of three determinations for each amino acid, it can be seen that *C. fatigans* extracts contain considerably greater quantities of aspartic acid, taurine, threonine, alanine, lysine, arginine, valine and the leucine-isoleucine complex, than extracts of *A. quadrimaculatus*.

In general, *Aedes aegypti* extracts also contain somewhat larger amounts of several amino acids than those of *A. quadrimaculatus* but less than *C. fatigans*. On the other hand, the *Anopheles* species exhibits a higher level of glutamic acid than either of the two culicine species. It will be noted that the total weights of free amino acids in the latter species are greater than in *Anopheles*.

Immediately after running a chromatogram and prior to spraying it with ninhydrin to visualize amino acids and other ninhydrin-positive substances, it may be examined under ultraviolet light in order to observe fluorescent materials.

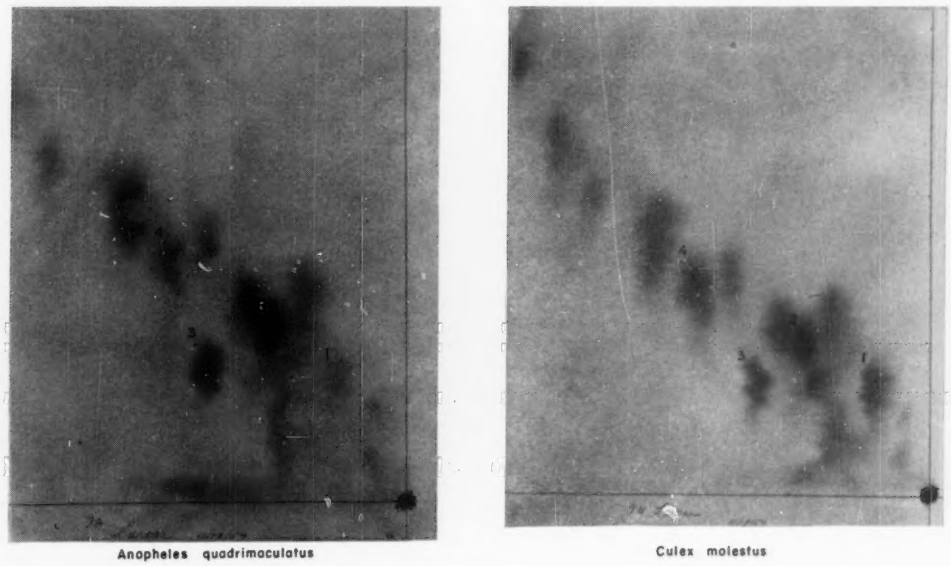

FIG. 3. Two-dimensional chromatograms of whole specimens of *A. quadrimaculatus* and *C. molestus* showing their characteristic amino acid-peptide patterns. Propanol-diethylamine was used as the first solvent (vertical) and butanol-acetic acid as the second (horizontal direction).

Figure 4 compares the patterns of the various yellow and blue-fluorescing spots seen in chromatograms of four different species of mosquitoes. It can be seen that each species exhibits a different pattern. In general, the fluorescing areas do not correspond with the spots which appear after spraying the chromatogram with ninhydrin. The former may be traced on another sheet of paper or marked by pencil on the chromatogram prior to spraying with ninhydrin.

Although vitamin A was not observed on chromatograms prepared with the various mosquito extracts, riboflavin was demonstrated in *C. molestus*, *C. fatigans*, *A. aegypti* and *A. quadrimaculatus*, and appeared as a yellow, fluorescent spot. The *Culex* species were found to contain significantly more riboflavin than the others.

DISCUSSION

The foregoing results demonstrate various paper chromatographic techniques which may be employed in comparative studies of insects. The method is highly adaptable to the particular need. The reproducibility of results with whole bodies and parts of insects makes it unnecessary to have elaborate equipment for extract preparation. One-dimensional chromatograms are ideal for comparative purposes. However, if identification of the chemical constituents is necessary, then two-dimensional chromatograms must be employed.

The results obtained from the spectrophotometric analysis of certain amino acids eluted from two-dimensional chromatograms of *C. fatigans*, *A. aegypti* and *A. quadrimaculatus* confirm and extend our previous findings (Micks and Ellis, 1951, 1952) of marked quantitative differences in free amino acid levels between genera. Although we have been also able to distinguish between several closely related species of a given genus on a quantitative basis (Micks, 1954), we have not observed intrageneric qualitative differences in amino acids as reported by Ball (1953) in three species of *Culex*. However, his work with *C. fatigans* from southern California, employing procedures essentially like those we had used (Micks and Ellis, 1951), completely confirms our chromatographic results with this same species from Texas.

Our findings with mosquitoes agree with those of Hadorn and Mitchell (1951) and Buzzati-Traverso (1953) with *Drosophila melanogaster*, as well as the results of Hadorn and Kühn (1953)

with *Ephestia kühniella*, as to the taxonomic value of patterns of fluorescent materials. These authors have shown that even if the amino acid-peptide composition of various strains is the same, differences in fluorescent substances may be quite marked. In fact, Buzzati-Traverso (1953) has been able to detect genotypic differences hidden under identical morphological features. It should be pointed out, therefore, that two sets of results may be derived from a single chromatogram without need for highly-specialized or expensive equipment. Visualization of the filter paper with ultraviolet light will provide one set of data and then spraying with ninhydrin will produce another. Also, it is important to use more than one solvent initially in order to determine which one provides maximum differentiation of the materials being compared.

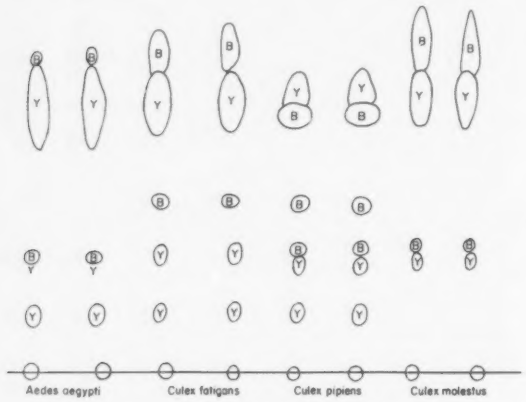

FIG. 4. Tracing of a chromatogram run in propanol-NH_3 showing the patterns of yellow (Y) and blue (B)-fluorescing spots as seen under ultraviolet light prior to treatment with ninhydrin.

Specimens to be compared chromatographically should be collected or kept under similar conditions if at all possible in order to obtain the most clear-cut results. In other words, starved specimens should be compared with starved specimens and those which have been fed should be compared with other fed species. It is generally not desirable to prepare chromatograms of insects for several days following a blood meal since blood components interfere with separation of the ninhydrin-positive spots.

Caution should be exercised in the interpretation of chromatographic results. There are several published reports wherein it was concluded that a number of amino acids were absent in certain insect species simply because they did not appear on the chromatogram. Most of these investigators have since learned that some amino acids must be present in quantities ten to twenty times those of others in order to be seen after spraying with a reagent. Therefore, one must apply increasing amounts of material to two-dimensional chromatograms until the maximum number of spots is seen. In this connection, it is interesting to note that some of the first studies involving the determination of amino acids in insects by paper chromatography list only eight or ten amino acids. Almost without exception, these amino acids are those which need to be present in only small quantities in order to be seen.

Paper chromatographic methods have been used to study the representatives of several orders such as the Odonata, the Orthoptera, the Hempitera, the Hymenoptera, the Lepidotera and the Diptera. The last-named order has been more extensively investigated than the others. Whereas the larger specimens lend themselves better to the analysis of certain components such as hemolymph, smaller insects, such as *Drosophila*, fleas, lice and mosquitoes are particularly suitable for amino acid or other studies on whole bodies.

TABLE I

AMINO ACID LEVELS OF ADULT FEMALE MOSQUITOES
(expressed in mg./gram)

AMINO ACID	*Aedes aegypti*	*Anopheles quadrimaculatus*	*Culex fatigans*
Aspartic acid	0.48	0.28	0.40
Glutamic acid	0.52	0.76	0.44
Taurine	0.54	0.48	0.56
Threonine	0.40	0.36	0.48
Alanine	2.20	1.00	2.12
Lysine	0.51	0.40	0.60
Glutamine	0.78	0.80	0.84
Arginine	0.72	0.66	0.90
Histidine	0.86	0.84	0.84
Proline	0.51	0.44	0.44
Valine	0.84	0.50	0.88
Leucines	1.14	0.82	1.36
Totals	9.50	7.34	9.86

As to the usefulness of amino acid patterns in systematics, it is of interest that there are eight amino acids which have been shown to be present in practically all insects studied to date. These are alanine, glutamic acid, glycine, isoleucine or leucine, proline, serine, tyrosine and valine. On the other hand, histidine, phenylalanine, alpha-amino-butyric acid, asparagine and hydroxyproline have been found to be constituents of only a few insects studied. Although there may be instances wherein closely-related species may be separated on the basis of qualitative differences in their amino acid compositions, it seems likely that most comparisons will be on a quantitative basis.

SUMMARY

Paper chromatographic methods were applied to whole specimens as well as extracts of Hemiptera (*Triatoma gerstaeckeri* and *T. infestans*), Orthoptera (*Periplaneta americana, Blattella ger-*

manica and *Supella supellectilium*) and Diptera (*Culex molestus, C. fatigans, C. pipiens, Aedes aegypti* and *Anopheles quadrimaculatus*).

One-dimensional chromatograms revealed qualitative differences between the three orders (Hemiptera, Orthoptera and Diptera) as to their respective patterns of ninhydrin-positive substances. Three genera of mosquitoes (*Culex, Anopheles* and *Aedes*) could be readily distinguished by marked quantitative differences in their amino acid levels.

Chromatograms of larvae and adults of *Culex molestus, C. fatigans* and *A. quadrimaculatus*, although similar, exhibited quantitative differences in their ninhydrin-positive materials. Both sexes presented essentially the same chromatographic patterns. Three species of *Culex* (*fatigans, pipiens* and *molestus*) as well as *Aedes aegypti* presented qualitatively different chromatograms of fluorescent substances.

Paper chromatography can be employed as a comparative tool to whole insect specimens or parts thereof without need for special equipment. Both the ninydrin-positive and the fluorescent patterns can be used in resolving taxonomic problems.

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INSECTS OF MICRONESIA HETEROPTERA: MIRIDAE, by José C. M. Carvalho. Insects of Micronesia Vol. 7, No. 1, pp. 1–100, 58 figs. 1956. Bernice P. Bishop Museum, Honolulu, Hawaii.

This is another of the growing list of contributions resulting from recent intensive studies conducted in Micronesia. The scope and objective of these studies have been described in 1954 by Linsley Gressitt in his introduction to the Insects of Micronesia series. Briefly, the objective is to make known the insect fauna of the scattered islands in the vast area known as Micronesia. So far as the Miridae are concerned, this objective seems to have been attained.

Dr. Carvalho had at his disposal collections made by military personnel during World War II as well as the large quantity of material obtained by Dr. Gressitt and other collectors working under the auspices of the Pacific Science Board and the Bishop Museum. From this material he has named one new subfamily, four new genera and 44 new species. All told, 87 species are reported from the area with only 15 being known from outside Micronesia. This fact combined with the discovery of a new subfamily and four new genera suggest a greater degree of endemicity than has previously been suspected for any of the families of Heteroptera found in these island groups. Each species is illustrated and keys for subfamilies, genera and species are provided with the result that Miridae from Micronesia can now be identified more readily than those from any other part of the world.

Reece I. Sailer.

THE EUROPEAN CORN BORER, PYRAUSTA NUBILALIS (HUBN.), AND ITS PRINCIPAL HOST PLANT

II. THE INFLUENCE OF NUTRITIONAL FACTORS ON LARVAL ESTABLISHMENT AND DEVELOPMENT ON THE CORN PLANT[1]

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The problem of identifying the factors underlying the host plant relationships of phytophagous insects is made up of many interrelated facets. Although there is a voluminous body of literature on the subject of insect-host plant relationships, there is a paucity of precise information. This situation has been caused by the various contributing aspects not having been adequately isolated for experimental analysis. Insect-plant adaptations are of sufficient biological and practical importance to merit more study than has been devoted to them in the past.

There have been many reports of experiments in which the effects of different host plants on the survival, growth, and reproduction of various phytophagous insect species have been determined. To cite the work of Hodge (1933), Seamans and McMillan (1935), Tauber *et al.* (1945), Brett (1947), and Snyder (1954) is to mention but a few such studies. Some very striking differences in host plant suitability have been recorded, but the factors underlying the apparent differences in host plant efficacy have never been determined. The usual conclusion drawn has been that the different host plants tested differed in the degree to which they satisfied the nutritional requirements of the experimental insects. If the term *nutritional requirements* is defined in its broadest sense to include all the conditions and factors necessary to both successful feeding and the biochemical adequacy of the diet, such a conclusion would seem warranted, even though accomplishing little more than to restate the problem of host plant specificity. In order to prevent confusion, the term *nutritional requirements* should be defined as referring only to the chemical factors essential to the adequacy of the ingested diet. Chemical factors important to normal feeding behavior should be described as the insect's *chemical feeding requirements*. The term *physical feeding requirements* should be used to define the insect's requirements as far as dietary texture, position, light intensities, and other physical factors influencing feeding behavior are concerned.

The suitability of a given plant host for a particular insect is at least partially dependent upon how well it meets these three phases of the insect's biological requirements. Other factors, such as the presence or absence of toxic substances in the plant tissue, the synchrony of the phenology of plant and insect, and the ovipositional behavior of the gravid female are also prominent in influencing host specificity.

From a physiological standpoint, our knowledge concerning insect-plant relationships is advanced but little by laboratory studies of the performance of insects on a wide variety of plants, unless there is also an elucidation of some of the factors which underly the differences observed. The conclusion drawn by Bottger (1940), that European corn borer larvae grew poorly on beet leaves because of the high sucrose content of such leaves, would seem unjustified in view of all the other physical and chemical differences that might exist between the tissues of beet plants and corn plants. Hocking (1953) had little basis for his conclusion that *Agrotis orthogonia* Morr. larvae grow better on living wheat seedlings than on excised leaves of wheat seedlings because of the higher nitrogen and water content of the living seedlings. Many more examples might be cited in which this type of experimental work was carried on under conditions where so many variables were left uncontrolled that the causes of observed differences in the suitability of the plant for the insect were not demonstrable. It cannot be assumed that, at ordinary temperatures, excised plant tissue is comparable to normal tissue for longer than an hour or so following its removal from the plant (Bonner 1950). In addition to biochemical degradation, excised plant tissues undergo changes in water and hydrogen ion relationships, and are subject to microbial attack which will effect further changes in the physical and chemical organization. The technique of rearing European corn borer larvae on excised corn leaves was used by Beck and Lilly (1949), but subsequently the method was abandoned as being too crude. Excised plant tissues have been used in many food plant studies, and the interpretation of the data is rendered difficult, if not impossible, by the serious sources of error inherent in such techniques.

Cannibalism is another source of error in food plant studies involving mass rearings of a phytophagous insect. The influence of this factor

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may be very striking, at least in the case of the European corn borer. Experiments in the writer's laboratory have shown that on a deficient diet, the average weight of larvae that have eaten one other larvae receiving the same diet may be more than three times as great as that of larvae reared in isolation.

Little experimental evidence exists which would support the hypothesis that differences in host plant adequacy reflect differences in the degree to which the plants meet the nutritional requirements of the insects tested. Nutritional factors contributing to host plant adequacy have been identified in a few instances, however. Pepper and Hastings (1943) found that the fecundity of the sugar beet webworm, *Loxostege sticticalis* (L.), was positively correlated with the linoleic acid content of the host plant tissue. Grison (1952) reported that fecundity and longevity of the female Colorado potato beetle, *Leptinotarsa decemlineata* (Say), were positively correlated with the lecithin content and negatively correlated with the glucose content of the leaves of the food plant. Since plant lecithins usually contain a high per cent of linoleic acid, (Bonner, 1950), there is an apparent similarity between these two reports. The work of Fraenkel and Blewett (1945, 1946) has established the importance of linoleic acid in the nutrition of some insect species. Wilson (1933) and other workers have found that wood high in starch content was much more favorable for powder post beetle infestation than was wood low in starch. Evans (1938) found population growth of the aphid *Brevicoryne brassicae* (L.) showed a positive correlation with the protein content of the host plant. The pea aphid, *Macrosiphum pisi* (Harris), was found to feed most successfully on those portions of the host plant containing the highest levels of sucrose (Emery, 1946). The few instances in which nutritional factors have been demonstrated to influence the host plant adequacy are not sufficient to establish any sort of a generalization concerning the relationship between an insect's nutritional requirements and its host specificity. They are sufficient, however, to indicate that nutritional aspects of insect-host plant relationships need further investigation.

An opposite and extreme view has been adopted by Fraenkel (1953). He advanced the theory that host plant specificity must be caused primarily by the insect's chemical feeding requirements, since all insects have essentially similar nutritional requirements. The plausibility of the theory rests, in part, on what is meant by the essential similarity of nutritional requirements. If only the general animal nutritional requirements are considered, the phytophagous insects will, undoubtedly, show a requirement for B vitamins, amino acids, lipids, inorganic ions and perhaps carbohydrates. Since insects are animal organisms, it would be very suprising to find otherwise. "Essential similarity" cannot be construed as being synonymous with "identical", as the different insects studied do not show identical nutritional requirements. For instance, *Tribolium confusum* Duv. does not require a dietary source of carbohydrate (Fraenkel and Blewett 1943a); whereas the diet of *Tenebrio molitor* L. must be about 80 per cent carbohydrate for normal growth (Fraenkel *et al.*, 1950). Both *Tenebrio* and *Tribolium* require carnitine (Fraenkel, 1951, French and Fraenkel, 1954), but most other insects do not require this substance in their diet (Fraenkel *et al.*, 1955). Besides a sterol, lepidopterous larvae of the genus *Ephestia* require a dietary source of linoleic acid, or other long chain unsaturated fatty acid, as well as an antioxidant such as alpha tocopherol (Fraenkel and Blewett, 1945). Larvae of the moth *Tineola bisselliella* (Hum.), on the other hand, were found to require no lipid other than a sterol (Fraenkel and Blewett, 1946b). Many other examples of specific differences among insect species might be cited, and there is no basis for postulating that significant differences will not be found among the great variety of plant feeding forms. Some of the relationships between differences in nutritional requirements among stored products insects and the insects' distribution on natural foods have been pointed out by Fraenkel and Blewett (1943b). Within the concept of "essential similarity" of the nutritional requirements of animals in general and insects in particular, many differences in qualitative and quantitative dietary requirements may exist which will help to explain at least some aspects of the complex relationships between an insect and its host plant.

In respect to the European corn borer and its principal host plant, the observations of many workers have pointed to the possible importance of nutritional influences on the performance of the larva on the corn plant. It is well established that small (late planted) corn is less infested by the first generation borer than large plants (early planted) (Barber, 1926; Cutright & Huber, 1928; Huber *et al.*, 1928; Patch, 1929; and many subsequent workers). Small corn plants are not only refractive to oviposition (Patch, 1929; Houser & Huber, 1929; Scholsberg & Mathes, 1937; and others) but also unsuitable for larval survival and growth (Ficht, 1931; Polivka & Huber, 1931; Kelsheimer and Polivka, 1936; Schlosberg and Baker, 1939; and many others). As the corn plants grow larger, they become progressively better suited as larval hosts, and in the papers cited above, the authors generally postulated that the host plant improved nutritionally as it reached the borer-susceptible pre-tasselling growth stage.

Host plant selection is a function of the gravid moth, and except for minor plant-to-plant migration, the borer larvae tend to remain on the

plant on which they hatch. The selection of the plant tissue on which to feed is a larval function, and the factors underlying their orientation and feeding behavior on the corn plant were discussed in detail in a previous publication (Beck, 1956b). The borer's physical feeding requirements are associated with its negative phototaxis and positive thigmotaxis. Its chemical feeding requirements were found to be represented mainly by the insect's saccharotrophism, although minor feeding responses were also elicited by protein and lipids (Beck, 1956a; 1956b).

The present study was undertaken in an effort to determine: (1) the role of larval nutritional requirements in the host plant inadequacy of small, or young, corn plants; and (2) the nutritional significance of larval saccharotrophism.

TABLE I.

BASAL PURIFIED DIET EMPLOYED IN STUDIES OF THE NUTRITIONAL REQUIREMENTS OF THE EUROPEAN CORN BORER.

CONSTITUENT	AMOUNT PER DIET (G)	CONCENTRATION IN DIET (MG/G WET WEIGHT)
Water	125.00	876.7
Bactoagar	3.35	23.5
Cellulose fiber	2.00	14.0
Glucose	5.00	36.0
Casein, Vitamin test	5.00	36.0
Cholesterol	0.22	1.5
Corn oil (with 1% α tocopherol)	0.22	1.5
Wesson Salts	0.44	3.1
Leaf Concentrate	1.00	7.0
B-Vitamin Mixture	0.10	0.7
Total	142.33	1000.0

METHODS

The methods used in this investigation have been described in detail in previous publications. The basal purified diet used in rearing experiments is shown in Table I. The aseptic rearing procedure described by Beck and Stauffer (1950) was employed. The method of determining growth curves by head width measurement was described previously (Beck, 1950). The technique and simplified purified diet used to measure larval feeding responses have also been described (Beck, 1956a). Plant parts which were analyzed for total sugars were treated as described by Beck (1956b).

RESULTS AND DISCUSSION

A series of experiments was designed to determine whether the high rate of mortality observed among newly hatched European corn borer larvae on very young corn plants could be explained on the basis of the nutritional inadequacy of the host plants. High larval mortality could be explicable on a nutritional basis only if the larvae were found to be extremely sensitive to deficiencies of one or more required nutrients. Using the purified diet and aseptic techniques previously developed, a series of diets displaying different extreme deficiencies was employed and the mortality of newly-hatched larvae receiving these diets was compared to the mortality of larvae feeding on leaves of corn.

Of the deficiencies tested, only the complete absence of nutrients resulted in a larval mortality comparable to that caused by leaves of corn seedlings (Table II). It is, of course, impossible that plant tissues could be naturally devoid of any of the principal nutrients required by the borer larvae. Thus, according to these results, the inadequacy of corn seedlings as borer host plants was not caused by a nutritional deficiency in the plant tissues.

TABLE II

MORTALITY OF NEWLY HATCHED LARVAE OF THE EUROPEAN CORN BORER UNDER DIFFERENT DIETARY CONDITIONS FOR 72 HOURS

DIETARY SUBSTRATE	LARVAE TESTED (NO.)	LARVAL MORTALITY (PERCENT)
Leaves of seedling corn	20	75
Leaves of corn in tassel stage	20	15
Purified diet, no nutrients	20	100
Purified diet, complete nutrients	17	6
Purified diet, no sugar	15	7
Purified diet, no protein	20	0
Purified diet, no corn leaf factor	20	0
Purified diet, no lipids	17	6
Purified diet, no B-Vitamins	20	30

The high mortality on leaves of corn seedlings and under starvation conditions (Table II—purified diet, no nutrients) suggests the possibility that leaves of the corn seedling contain a feeding deterrent, resulting in larval starvation. Feeding response experiments have shown that such is not the case, however. Dietary media containing homogenized seedling leaves elicited a more intense feeding response than did control diets containing no plant tissue. At the end of the 24 hour experimental period, 69 percent of the larvae were feeding on the diet containing the leaf tissue, and 31 percent were feeding on the control diet.

Under the experimental condition employed, no evidence was obtained that the unsuitability of young corn plants for borer establishment is related to either a nutritional deficiency or an adverse effect on larval feeding behavior.

In the first paper of this series (Beck, 1956b) the shifting of borer feeding sites on the corn plant was discussed in detail, and it was shown that the feeding behavior could be accounted for on the basis of developmental changes of the

plant and larval behavior characteristics. The behavior responses involved were found to be: 1) negative phototaxis; 2) positive thigmotaxis; and 3) positive saccharotrophism. Saccharotrophism was defined as a tendency for an organism to concentrate its feeding on substances high in sugar content, and some of the characteristics of saccharotrophism in borer larvae have been demonstrated (Beck, 1956a; 1956c). The possible importance of nutritional factors in the borer's shifting feeding sites as it and the plant mature is in need of experimental clarification. A beginning in this direction has been made in a study of the borer's nutritional requirements for sugar and protein. The importance of sugar and protein in the nutrition of the European corn borer has been demonstrated previously when it was shown that both are required for normal growth and development (Beck *et al.*, 1949; Beck, 1950). The present study was undertaken to determine whether the requirements undergo changes during the insect's development and, if so, whether such changes can be correlated with the feeding habits of the larvae.

In this group of experiments, the dietary levels of glucose and casein were systematically varied. All other constituents of the diet were, of course, held constant at the optimal concentrations. The larvae did not require glucose for normal growth during the early larval stadia, as the head capsule widths of larvae reared on diets containing different amounts of glucose showed no significant differences (Table III). It was shown in an earlier paper that head capsule width is dependent on body weight (Beck, 1950). Highly significant differences in body weight were observed at maturity and in the per cent attaining pupation at 21 days of age. It is apparent that dietary sugar was required by the late instars. A level of about 18 milligrams per gram of medium resulted in apparently optimum growth and development.

Protein is essential for growth and development (Table IV). The minimum casein concentration required to effect normal growth was about 18 milligrams per gram of dietary medium. No growth occurred in the absence of casein, and growth was severely limited at 7 milligrams per gram. In experiments in which glucose and

TABLE III

EFFECT OF DIETARY GLUCOSE CONCENTRATION ON THE GROWTH AND DEVELOPMENT OF EUROPEAN CORN BORER LARVAE

GLUCOSE	AVERAGE HEAD CAPSULE WIDTH			AVERAGE WEIGHT AT 21 DAYS	PUPATION AT 21 DAYS
	2ND INSTAR	3RD INSTAR	4TH INSTAR		
(MG/G DIET)	(MM)	(MM)	(MM)	(MG)	(%)
0.0	0.47	0.79	1.21	60.3**	25**
7.0	0.45	0.78	1.28	68.8**	50**
18.0	0.47	0.78	1.20	98.4	92
36.0 (control)	0.46	0.80	1.26	98.1	100
54.0	0.49	0.78	1.24	94.8	100

**Difference from control significant at the 0.01 level of probability.

TABLE IV

EFFECT OF DIETARY CASEIN CONCENTRATION ON THE GROWTH AND DEVELOPMENT OF EUROPEAN CORN BORER LARVAE

CASEIN	AVERAGE HEAD CAPSULE WIDTH			Average WEIGHT AT 21 DAYS	PUPATION AT 21 DAYS
	2ND INSTAR	3RD INSTAR	4TH INSTAR		
(MG/G DIET	(MM)	(MM)	(MM)	(MG)	(%)
0.0	(no growth or survival)	———	———	———	———
7.0	0.42*	0.66**	0.95**	55.3**	0**
18.0	0.46	0.75*	1.21	91.1	100
36.0 (control)	0.46	0.80	1.26	100.3	100
54.0	0.47	0.80	1.27	101.2	100

*Difference from control significant at the 0.05 level of probability.
**Difference from control significant at the 0.01 level of probability.

casein were varied simultaneously but always employed in a 1:1 ratio, larval growth data were comparable to those of Table IV.

Experiments in which the dietary levels of glucose and casein were changed midway in the larval growth period yielded the results shown in Table V. These data clearly show that the larval requirement for glucose was low during the early growth stages (instars 1 to 3) and high during later development (instars 4 and 5). The requirement for casein, on the other hand, was high during the early larval development and low during the late stages. These results are fully consistent with those shown in Tables III and IV.

The finding that the larval requirements for protein and sugar change during the larval growth period is very significant to the study of the host plant relationships of this insect. On the basis of plant analyses and the results obtained in the experiments discussed above, the nutritional adequacy of several corn plant tissues was estimated (Table VI). The estimated nutritional values appear to correlate well with the biology of the borer on the corn plant. Initial larval feeding is predominantly on leaf tissue, which is adequate for early larval growth. Leaf tissue is inadequate for late larval growth, but under natural conditions the borer does not normally feed on leaf tissue beyond the late second or early third instar. The developing tassel is normally invaded by young larvae of the first generation, and some of these larvae may remain in tassel tissue for the remainder of their growth period. Tassel tissue and pollen is high in both sugar and protein, and is nutritionally adequate for all stages of larval development. Borer

TABLE V

EFFECT OF CHANGING DIETARY LEVELS CASEIN AND GLUCOSE ON THE GROWTH AND DEVELOPMENT OF EUROPEAN CORN BORER LARVAE

DIET DURING DAYS 1–10		DIET DURING DAYS 11–20		AVERAGE WEIGHT AT 20 DAYS	PUPATION AT 20 DAYS
CASEIN	GLUCOSE	CASEIN	GLUCOSE		
(MG/G DIET	(MG/G DIET)	(MG/G DIET)	(MG/G DIET)	(MG)	(%)
30	30	30	30 (control)	98.1	100
53	7	53	7	60.2**	40**
53	7	7	53	123.7*	100
7	53	7	53	12.8**	0**
7	53	53	7	30.5**	0**

*Difference from control significant at the 0.05 level of probability.
**Difference from control significant at the 0.01 level of probability.

TABLE VI

SUGAR AND PROTEIN CONTENT OF CORN PLANT TISSUES IN RELATION TO THE NUTRITIONAL REQUIREMENTS OF EUROPEAN CORN BORER LARVAE

PLANT TISSUE	TYPICAL AMOUNTS NUTRIENT IN FRESH TISSUE		ESTIMATED NUTRITIONAL ADEQUACY FOR EUROPEAN CORN BORER	
			FOR EARLY LARVAL GROWTH	FOR LATE LARVAL GROWTH
		MG/GM.)		
leaves	sugars	10	adequate	deficient
	proteins	36[a]	adequate	adequate
leaf sheath	sugars	18	adequate	adequate
	proteins	6[a]	deficient	adequate
internode	sugars	33	adequate	adequate
	protein	8[b]	deficient	adequate
green tassel	sugars	31	adequate	adequate
	protein	29[a]	adequate	adequate
pollen	sugars	52[c]	adequate	adequate
	protein	98[c]	adequate	adequate
ear shank	sugars	30	adequate	adequate
	proteins	8[a]	deficient	adequate
kernels	sugar	36[b]	adequate	adequate
	proteins	30[b]	adequate	adequate

[a]Calculated from Whitehead *et al.* (1948.
[b]From Bottger (1951.
[c]From Haber & Gaessler (1942).

larvae invade leaf sheath, internode, and earshank tissues fairly late in larval development, and these tissues are of nutritional adequacy only for the later larval instars. Pollen and kernals are well balanced in respect to sugar and protein and represent adequate diets for larval development, throughout the borers' growth period.

The saccharotrophic behavior response of borer larvae does not appear to be correlated with a nutritional requirement for sugars during the early larval stages. It may, however, be instrumental in orienting the larvae to tissues containing the high sugar levels required by the late developmental stages. The sugar content of the plant tissues does not seem to act as a token stimulus orienting the young larvae to tissues high in protein, as there appears to be no correlation between the sugar and protein contents of the different tissues of the corn plant.

SUMMARY

1. Nutrition and feeding response experiments with newly-hatched European corn borer larvae on variously deficient purified diets failed to support the hypothesis that nutritional deficiencies in the host plant tissue are responsible for the high mortality observed among borer larvae feeding on the leaves of corn seedlings.

2. The requirement for sugar (glucose) was negligible during the early stages of larval growth, but high during the late larval development.

3. The nutritional requirement for protein (casein) was high during early larval growth and low during late larval development.

4. The larval requirements for dietary sugar and protein were correlated with the normal biology and feeding behavior of the borer on corn plants.

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THE MODE OF ACTION OF ALDRIN AND DIELDRIN IN PERIPLANETA AMERICANA (L.)[1]

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Although aldrin and dieldrin have been widely used as insecticides for several years, little significant information has been developed concerning their mode of action in insects. It is the purpose of this paper to develop information in this regard, particularly as to their comparative toxicities, sites of toxic action, and metabolism in the American cockroach, *Periplaneta americana* (L.).

MATERIALS AND METHODS

The aldrin m.p. 98–100° C. and dieldrin m.p. 173° C. which were used in these experiments were funished by the Agricultural Chemicals Division of the Shell Chemical Corporation, Denver, Colorado, and were recrystallized several times from ethanol. Individual 1λ doses in acetone were applied topically by a micrometer-driven syringe (March & Metcalf 1949) to the dorsum of the thorax beneath the wings, or were injected ventrally through the intersegmental membrane between the third and fourth segments of the abdomen. The studies on symptomatology and site of action were based on the work of Tobias and Kollros (1946) with DDT and the procedures used were generally analogous. Carbon dioxide anesthesia was used to immobilize the insects.

An ascending paper-chromatographic method was developed for the separation of aldrin and dieldrin. Tapered strips of Whatman No. 1 filter paper, 205 mm. in length, 24 mm. in width at the top, and 18 mm. at the bottom were impregnated by immersion in a 10 percent (weight by volume) solution of Silicone 550 (Dow Corning Corporation, Midland, Michigan) in petroleum ether (Skellysolve B) and allowed to dry on paper toweling in the air. A solution of glacial acetic acid in water (3 to 1 by volume) was used as the mobile solvent. Solutions of aldrin or dieldrin or extracts of treated roaches were applied by capillary to a minimum area 1.5 cm. from the base of the paper strip in preparation for chromatographing. After the development of the chromatogram, the paper strip was air dried for about 15 minutes, cut into sections 0.75–1.5 cm. in length, and diced. The pieces from each section were shaken for 5 minutes in 1 ml. of acetone and the acetone extract added to 100 ml. of water containing 20 third instar larvae of the southern house mosquito, *Culex quinquefasciatus* Say. Mortality was determined after 24 hours. This procedure located the areas containing toxic materials and was sensitive to about 1γ of aldrin or dieldrin. Aldrin was also indentified by spraying the paper with a 0.1 percent solution of potassium permanganate, which decolorized by reaction with the double bond. These technics produced good separations of aldrin

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and dieldrin, which had approximate R_f values of 0.2 and 0.5, respectively.

Chemical determinations of aldrin were carried out by coupling with phenyl azide to form aldrin phenyldihydrotriazole which when acidified, diazotized, and coupled with dinitroaniline gives an intense red color (Danish and Lidov, 1950; O'Donnell *et al.*, 1954). This reaction is not given by dieldrin unless this compound is first converted to aldrin by formation of the bromohydrin and reduction (Shell, 1953). Thus the reaction may be made selective for either compound.

TABLE I

TOXICITY OF ALDRIN AND DIELDRIN TO ADULT FEMALE AMERICAN COCKROACHES

Compound	Dosage γ/gm.	Percent Mortality After Following Intervals in Hours				
		24	48	72	96	120
Topical Application						
Aldrin	1.0	0[a]	0	10	15	20
	1.4	0	0	15	45	50
	2.2	0	5	25	50	50
	3.0	0	10	40	60	70
						LD_{50}—1.9
Dieldrin	1.0	5	5	10	25	35
	1.4	0	5	35	45	50
	2.2	0	25	45	65	85
	3.0	0	40	65	80	95
						LD_{50}—1.3
Injection						
Aldrin	1.0	0	5	10	20	30
	1.4	15	30	45	45	50
	2.2	20	30	55	65	65
	3.0	40	65	75	80	80
						LD_{50}—1.5
Dieldrin	1.0	10	30	30	40	45
	1.4	0	30	60	65	65
	2.2	55	80	85	85	85
	3.0	70	85	85	85	90
						LD_{50}—1.1

[a]Average of 2 series of experiments with 10 roaches at each dosage.

The fat storage of aldrin and dieldrin was studied by homogenizing the fat bodies of 5 to 10 roaches in 3 ml. of water, extracting the homogenate three times with 3 ml. of petroleum ether (Skellysolve B), and evaporating the combined extracts to leave an oily residue. This residue was re-extracted three times with 1-ml. portions of acetonitrile, 3 ml. of water were added to the combined acetonitrile extracts, and the mixture was re-extracted three times with petroleum ether. The residue obtained upon evaporation of the combined petroleum ether extracts was dissolved in 50 λ of acetonitrile and applied to the Silicone-treated paper strips, taking care to avoid any traces of oil, which decreased the separation on the chromatogram.

EXPERIMENTAL RESULTS

Toxicity.—The data in Table I show that the 120-hour LD_{50} values in γ/g. for aldrin and dieldrin to female American cockroaches were by topical application 1.9 and 1.3, respectively, and by injection 1.5 and 1.1. These values are in good agreement with the topical values of 1.0 and 1.5 reported by Kearns *et al.* (1949). The nearly equivalent toxicity values obtained both by topical and injection technics indicate that these insecticides are efficiently absorbed and transported to the site of action. This has been similarly demonstrated with DDT and lindane (Savit *et al.*, 1946). The data also show that dieldrin acts more rapidly than aldrin.

Symptomatology.—The symptoms induced in *Periplaneta americana* by aldrin and dieldrin poisoning appear to be identical. As is the case with DDT (Tobias and Kollros, 1946), they are initiated by a hyperextension of the legs, elevation of the center of gravity, and development of postural instability. At this stage the cockroach is sluggish and does not readily respond to stimuli. As hyperextension decreases the instability increases and the insect becomes more active, exhibiting, upon minor tactile stimulus, hyperactivity by running and falling on its back with violent convulsions of the legs. After a series of convulsions the roach becomes almost quiescent and then the legs again gradually show some movements which increase slowly until a new discharge of violent impulses is observed, followed by immobility. This picture is repeated until the insect dies. When completely prostrated, the cockroach will respond to a light touch of the antennae by a convulsion of all the legs. During the development of the symptoms an increase in the rate of heartbeat was observed. In contrast to DDT, there were no continuous movements of the legs represented by high frequency tremors. It appears that all these symptoms are caused by a stimulation of the central nervous system, and this is in accord with the picture of spontaneous electrical activity produced in the central nervous system of *Periplaneta americana* by aldrin and dieldrin poisoning (Lalonde and Brown, 1954).

Physiological Action.—a) *Decapitation.*—Groups of 10 roaches were decapitated and after 3 to 4 hours were injected with 3 γ/gm. of aldrin or dieldrin. The results in Table II illustrate the influence of decapitation on the production of paralysis. It should be noted that no differences could be detected in the action of aldrin and dieldrin. Although the symptoms produced were similar in both intact and decapitated roaches, the convulsions were not as violent in the decapitated specimens. It appeared that with the intact central nervous system the convulsions

were stronger and more frequent. Decapitation performed after posioning had become established had no apparent effect on the development of symptoms.

b) *Severance of the nerve cord.*—The longitudinal connectives of groups of 20 to 25 roaches were severed by cutting above and below the mesothoracic ganglion. After 5 to 8 hours 3 γ/gm. of aldrin or dieldrin in acetone were injected into each roach. From a group of 24 roaches injected with acetone alone, 5 were dead and 5 had the meso- and metathoracic legs paralyzed 48 hours after the operation, but in 14 specimens the reflexes of all legs were normal, though showing incoordination of the meso- and metathoracic legs when walking. No convulsions were observed. From 20 insects injected with aldrin, 7 were dead and 3 showed paralysis of the meso- and metathoracic legs after 48 hours, but in 10, convulsions occurred in all the legs. In a similar group of 26 roaches treated with dieldrin, 8 were dead and 8 exhibited paralysis,

TABLE II

INFLUENCES OF DECAPITATION ON THE SYMPTOMS PRODUCED BY ALDRIN AND DIELDRIN

COMPOUND	NO. OF ROACHES	OPERATION	PERCENT PARALYZED IN	
			24 HOURS	48 HOURS
Dieldrin	20	Decapitated	60	85
Dieldrin	10	Normal	60	80
Aldrin	20	Decapitated	30	55
Aldrin	10	Normal	30	80
Acetone	20	Decapitated	0	5

but in 10, symptoms occurred in all the legs. Severance of the nerve cord in roaches previously injected with aldrin or dieldrin and showing the characteristic symptoms of poisioning gave the same results. Thus the experiments demonstrate that severance of the nerve cord does not change the general picture of the symtomatology. Tobias and Kollros (1946) obtained similar results with DDT. As in the decapitation experiments, the convulsions were not so violent and did not occur with the same frequency in the operated insects as in the intact insects. In addition, convulsions were observed at times in one pair of legs and not in the others.

c) *Severance of leg nerves.*—In groups of 10 roaches the nerves of the mesothoracic legs were cut in such a way that this pair of legs was isolated from its central ganglion. After 24 hours aldrin, dieldrin, and DDT were injected into these roaches. With aldrin and dieldrin the mesothoracic legs remained completely paralyzed while the other legs showed typical symptoms of poisoning (see Table III). With DDT, however, the mesothoracic legs also showed symptoms. The same results were obtained with roaches poisoned before isolation of the legs from the central ganglion. These experiments demonstrate the absence of a peripheral effect and suggest that the ganglia of the ventral nerve cord may be the primary loci of action.

In order to develop further information on the essentiality of the ganglia for the production of symptoms, experiments were carried out using

TABLE III

INFLUENCE OF SEVERING NERVES TO MESOTHORACIC LEGS ON THE SYMPTOMS PRODUCED BY ALDRIN, DIELDRIN, AND DDT

COMPOUND	DOSAGE γ/gm.	NO. OF ROACHES	LEGS WITH SYMPTOMS
Aldrin	10	10	Pro- and metathoracic
Dieldrin	10	10	Pro- and metathoracic
DDT	130	10	All legs; mesothoracic with reduced tremors
Acetone	—	10	No symptoms; mesothoracic paralyzed

TABLE IV

SYMPTOMS DUE TO TREATMENT WITH ALDRIN, DIELDRIN, AND DDT, OBSERVED IN ISOLATED ROACH SEGMENTS CONTAINING A CENTRAL GANGLION, NERVES, AND LEGS

NO. OF SEGMENTS PREPARED	SOURCE OF SEGMENTS	POST-OPERATIVE TREATMENT	SYMPTOMS OBSERVED
36	Roaches poisoned with aldrin 10 γ/gm.	None	28 preparations with movements
29	Roaches poisoned with dieldrin 10 γ/gm.	None	17 preparations with movements
10	Roaches poisoned with DDT 130 γ/gm.	None	All with tremors
19	Normal roaches	Aldrin 10 γ	All with movements
20	Normal roaches	Dieldrin 10 γ	All with movements
15	Normal roaches	DDT 100 γ	All with tremors
15	Normal roaches	Acetone 1 λ	No movements

amputated legs and isolated roach segments containing a ganglion, nerves, and legs. Such preparations, if kept moist, were viable for 6 to 8 hours. In the amputated legs aldrin, dieldrin, or DDT was injected into the cut end of the leg. In the isolated segments these insecticides were injected into the vicinity of the ganglion. The data in Tables IV and V show that poisoning with aldrin and dieldrin produced convulsions in

legs with intact innervation to the central ganglia. However, no movements were observed in the legs amputated from roaches previously poisoned with aldrin and dieldrin at dosages as high as 10 γ/gm. or in legs amputated from normal roaches and then injected with either insecticide. In contrast, however, DDT at 130 γ/gm. produced symptoms, when injected into both isolated segments and amputated legs, as had been previously demonstrated by Tobias and Kollros (1946).

It is well known that nicotine in low concentrations blocks synaptic transmission of insects centrally as well as peripherally but does not affect axonal transmission (Libet and Gerard, 1938; Pringle, 1939). When 0.20 to 0.25 γ of nicotine in insect Levy's solution (Yeager, 1939) was injected into the vicinity of the ganglion in an isolated roach segment, there was a violent hyperactivity of the legs, followed by complete paralysis. After 20 to 30 minutes the nicotine was detoxified and the movements of the legs became normal. When the same experiment was run with segments isolated from roaches previously treated with aldrin or dieldrin and showing typical convulsions, there was a short period of activity followed by complete paralysis of the legs. After the detoxification of the nicotine the symptoms produced by aldrin and dieldrin reappeared.

TABLE V

SYMPTOMS DUE TO TREATMENT WITH ALDRIN, DIELDRIN, AND DDT OBSERVED IN AMPUTATED ROACH LEGS

No. of Legs Prepared	Source of Legs	Post-operative Treatment	Symptoms Observed
21	Roaches poisoned with aldrin 10 γ/gm.	None	No movements
35	Roaches poisoned with dieldrin 10 γ/gm.	None	No. movements
28	Roaches poisoned with DDT 130 γ/gm.	None	16 with tremors
24	Normal roaches	Dieldrin 10 γ	2 with movements
24	Normal roaches	Aldrin 10 γ	2 with movements
44	Normal roaches	DDT 100 γ	30 with movements
36	Normal roaches	Acetone 1 λ	4 with movements

From these experiments it can be concluded that aldrin and dieldrin have the same general convulsant action and that in both cases the ganglia of the ventral nerve cord are critically involved. Even high concentrations of 10 times the LD_{50} of those insecticides did not produce any evidence of direct action on the peripheral nerves or muscles. Since the symptoms of aldrin and dieldrin poisoning do not occur in legs whose nerves have been cut or in amputated legs, it appears that these insecticides do not produce their effects on structures peripheral to the central ganglion.

Storage of Dieldrin in the Fat Body of Roaches Treated With Aldrin.—Davidow and Radomski (1953) have shown that heptachlor fed to dogs and rats is stored in fatty tissues as heptachlor epoxide (1, 4, 5, 6, 7, 8, 8-heptachloro-2, 3-epoxy-4, 7-methanoindane). Therefore, by analogy it seemed possible that aldrin might be converted biologically to its corresponding epoxide, dieldrin. The paper-chromatographic technic and bioassay with mosquito larvae already described were used to investigate this aspect of the study. Less than 1 γ of aldrin or dieldrin could be separated and detected in this manner (see Tables VI and VII). When the paper chromatographs of pure aldrin or dieldrin were divided into 0.75 cm. sections and bioassayed it was found, as shown in Table VII, that the aldrin was located in sections 2 to 4, and the dieldrin in sections 6 to 9 of the chromatogram. If the chromatographic procedure was carried out with the extract of the fat bodies of 10 roaches to which 5 γ of aldrin and dieldrin had been added, the aldrin was located in sections 2 to 4 and dieldrin in sections 6 to 9. When aldrin alone was added to the extract there was activity only in sections 2 to 4 and none in the other sections which were bioassayed. Chromatograms of extracts prepared from roaches topically treated 48 hours previously with 8 γ/gm. of aldrin exhibited activity in sections 2 to 4 and 6 to 10 of the paper strips, while experiments with roaches treated with 3 γ/gm. of dieldrin showed activity only in sections 5 to 9.

TABLE VI

TOXICITY OF ALDRIN AND DIELDRIN TO THIRD INSTAR LARVAE OF THE SOUTHERN HOUSE MOSQUITO

Dosage γ/100 ml. of Water	Percent Mortality After 24 Hours	
	Aldrin	Dieldrin
0.50	18[a]	12[a]
0.75	60	38
1.00	98	82
1.50	100	95
	LD_{50}—0.65	LD_{50}—0.78

[a]Average of two series of experiments with 20 larvae at each dosage.

Supporting evidence for the transformation of aldrin to dieldrin was obtained using the colorimetric phenyl azide technic as adapted for dieldrin as well as aldrin by O'Donnell *et al.* (1954).

TABLE VII

BIOASSAY WITH THIRD-INSTAR MOSQUITO LARVAE OF CHROMATOGRAPHICALLY SEPARATED ALDRIN AND DIELDRIN, ALDRIN- AND DIELDRIN-FORTIFIED EXTRACTS OF ROACH FAT BODIES, AND EXTRACTS OF FAT BODIES OF ALDRIN- AND DIELDRIN-POISONED ROACHES

SECTION NO.	DISTANCE FROM ORIGIN, CM.	PERCENT LARVAL MORTALITY AFTER 24 HOURS				
			FORTIFIED FAT BODY		EXTRACTED FAT BODY OF ROACHES POISONED WITH	
		5 γ ALDRIN + 5 γ DIELDRIN	5 γ Aldrin + 5 γ DIELDRIN	5 γ ALDRIN	8 γ/gm. ALDRIN	3 γ/gm. DIELDRIN
1	0 –0.75	0	0	0	0	0
2	0.75– 1.50	100	20	100	40	0
3	1.50– 2.25	100	100	100	70	0
4	2.25– 3.00	100	50	48	15	0
5	3.00– 3.75	0	0	0	0	80
6	3.75– 4.50	100	100	0	50	100
7	4.50– 5.25	100	100	0	100	100
8	5.25– 6.00	100	100	0	100	100
9	6.00– 6.75	100	80	0	100	90
10	6.75– 7.50	0	0	0	30	0
11	7.50–15.00	0	0	0	0	0

Control studies in which 10 γ of dieldrin were added to the pooled fat bodies of five roaches before extraction and chromatography, gave separate recoveries in three replicates of 4.0, 2.0, and 5.0 γ of dieldrin on sections 6 to 9 of the chromatogram. Similar experiments were carried out with the extracts of the fat bodies from five roaches 48 hours after the topical application of 2 γ of aldrin to each. In three replicates the chemical determinations for aldrin on sections 5 to 9 of the chromatograms were completely negative although these sections were highly toxic to mosquito larvae. The chemical determinations for dieldrin on sections 5 to 9 gave recoveries of 3.8, 2.0, and 1.0 γ and the chemical determinations for aldrin on sections 1 to 4 gave recoveries of 2.6, 2.0, and 8.0 γ. Although appreciable quantities of aldrin and dieldrin were lost in the extraction process, the data provide additional evidence that aldrin is converted to dieldrin in the American cockroach.

SUMMARY AND CONCLUSIONS

Preliminary studies were carried out on the mode of action of aldrin and dieldrin in *Periplaneta americana* (L.). The compounds were nearly equitoxic and the respective 120-hour LD_{50} values were: by topical application, aldrin 1.9, dieldrin 1.3 γ/gm.; by injection, aldrin 1.5 and dieldrin 1.1 γ/gm. This indicates that the insecticides are very efficiently absorbed and transported to the site of action.

The symptoms of poisioning produced by aldrin and dieldrin arise from stimulation of the central nervous system and are antagonized by the action of nicotine. Unlike DDT, aldrin and dieldrin do not produce symptoms in isolated legs, indicating an absence of activity on structures peripheral to the central ganglion.

Experiments using paper chromatography, bioassay with mosquito larvae, and chemical determination of aldrin and dieldrin by the phenyl dihydrotriazole method, indicate that aldrin administered to roaches is partially converted to dieldrin.

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A CONTRIBUTION TOWARD A MONOGRAPH OF THE SCATOPSIDAE (DIPTERA). PART VI. THE GENERA SCATOPSE GEOFFROY AND HOLOPLAGIA ENDERLEIN[1]

EDWIN F. COOK

The genus *Scatopse* was first described by Geoffroy in 1762. This name has subsequently been accepted and used by all workers in the field. However, at the 1948 Session of the International Commission of Zoological Nomenclature at Paris, all of Geoffroy's 1762 names were declared unavailable. In 1954 a request was made that the Commission use its plenary powers to preserve the generic names in Geoffroy (1762) in the order Diptera (Stone, *et al.*, 1954). Since this seems to be a reasonable and logical request, it is here assumed that it will be granted.

Prior to Enderlein's first revisional study in 1912, the genus *Scatopse* included all species belonging to the subfamily Scatopsinae of the Bibionidae with the exceptions of *Corynoscelis eximia* Bohem., *Arthria analis* Kirby, and *Aspistes berolinensis* Meigen. Enderlein restricted the genus to include only *S. notata* L. (the type species) and *S. leucopeza* Mg. (in 1936 he indicated that there are three species in the genus but did not name the other one). Enderlein's restriction has not been followed by Duda (1928) nor by Edwards (1925). Duda sank *Rhegmoclema*, *Swammerdamella*, *Holoplagia*, *Colobostema* and *Reichertella* as synonyms. Séguy (1940) has, on the other hand, accepted the restriction of *Scatopse* and all of Enderlein's genera, but he has made a number of inexplicable alterations. For example, Séguy includes *Colobostema triste* (Zett.) in *Scatopse* but places its very near relative *C. nigripenne* (Mg.) in *Reichertella*. In addition, he designates *Scatopse pulicaria* Loew as the type species of *Reichertella*, although Enderlein clearly states that the type is *Scatopse femoralis* Mg.

The genera dealt with in the preceding papers in this series have been reasonably clear cut and distinct from one another. The remaining species in the Scatopsinae (exclusive of those in *Holoplagia*, *Psectrosciara*, *Ectaetia* and *Anapausis*) available to me are rather difficult to place. In addition to *Scatopse* and *Holoplagia* there are two additional generic names of Enderlein that might be used or must at least be accounted for. The first of these is *Reichertella*. For this Enderlein designated *Scatopse femoralis* Mg. as the type. However, according to Duda (1928) all specimens in the Zoologische Museum der Humboldt Universität in Berlin which were determined by Enderlein as *S. femoralis* are actually *S. nigra* Mg. Duda had seen the types of *S. nigra* and *S. femoralis* in Paris so his determination should be correct. Furthermore, according to Duda, *S. femoralis* Mg. is *S. notata* L. If *S. femoralis* is accepted as the type species of *Reichertella*, then that name is an objective synonym of *Scatopse*. If, on the other hand, *S. nigra* is accepted as the type of *Reichertella*, it is possible that the name might be retained or would be no more than a subjective synonym of *Scatopse*. The latter viewpoint is here adopted pending a decision by the Commission of Zoological Nomenclature. *S. nigra* is regarded as the type of *Reichertella* and, for the reason noted below, *Reichertella* is treated as a synonym of *Scatopse*. The other available name is *Rhaeboza* for which Enderlein designated *S. fuscipes* as the type. He did not indicate what other species should be included, but Séguy includes *S. subnitens* Verrall.

When one compares *S. nigra*, *S. fuscipes*, *S. notata* and the remaining unassigned species in this group anatomically, it can only be concluded that these are either all congeneric or that several additional monotypic genera must be described for some rather aberrant forms. Rather than unnecessarily multiplying generic names on the rather limited number of species available, it seems best at present to retain all of these in *Scatopse* and to sink *Reichertella* and *Rhaeboza* as synonyms. This makes *Scatopse* rather an unnatural group and certainly subject to revision as more material becomes available. The greatest need here is for more European species of which there are some twenty-five that I cannot assign to any genus or species group without examination.

In the present paper the following species will be included in the genus *Scatopse*: *S. notata* (L.), *S. lapponica* Duda, *S. flavocincta* Duda, *S. uncinata* (Melander), *S. collaris* (Melander), *S. fuscipes* Mg., *S. nigra* Mg., five hitherto undescribed species from North America and one from China. These are all described or redescribed here. It is impossible without examination of authentic specimens to decide what other previously described non-American species should be included here.

The genus *Scatopse* can be divided into three species groups anatomically. The *Notata* group, including *S. notata* (L.) (cosmopolitan), *S. lapponica* Duda (Europe and North America) and two new species, *S. alpestris* (North America) and *S. chinensis* (China), are all very closely

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related and have all frequently been identified as *S. notata*. The second group will be called the *Collaris* group. This includes *S. collaris* (Mel.), *S. uncinata* (Mel.), two new species, *S. producta* and *S. fatligi* from North America, and *S. flavocincta* Duda from Europe. The third group is the *Fuscipes* group with *S. fuscipes* Mg. (cosmopolitan) and *S. nigra* Mg. (Europe). There are in addition two new species which show affinities with the several groups above. *S. brevipalpis* (North America) is apparently intermediate between the *Fuscipes* and *Collaris* groups and *S. montana* (North America) shares characters with all three groups.

Only the biology of *S. notata* and *S. fuscipes* are known to any extent, and a brief discussion will be included in the description of those species.

The other genus treated in this paper is *Holoplagia* Enderlein (1912). There is a problem of nomenclature in regard to this also. Enderlein designated as the type *S. transversalis* Loew, but specimens determined as *S. transversalis* by Enderlein are actually *S. lucifuga* Loew. This error was originally noted by Duda (1928). I have had available a male specimen of *S. lucifuga* Loew from Berlin (through the courtesy of Dr. Fritz Peus) which has a type label. I have also had one male and two females determined as *S. transversalis* by Enderlein, and the latter are clearly the same as the type of *S. lucifuga*. I have seen no specimens of *S. transversalis*, but according to Loew's original description and the description of Duda this is very close to *S. lucifuga* and is probably congeneric. Consequently, no problem would be encountered even if the type species were changed. *S. lucifuga* will, however, be considered the type of *Holoplagia*.

In this paper *H. lucifuga* will be redescribed as will *H. guamensis* Johannsen. These are the only species in this genus available to me. From available descriptions it appears that at least eight other species should be included in this genus. These will be cited under the discussion of the genus.

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Scatopse Geoffroy

Scatopse Geoffroy, 1762, Hist. des Insectes, 2: 544; Meigen, 1818, Syst. Besch. 1: 233; Loew, 1846, Linnaea Entomologica, 1: 324; Zetterstedt, 1850, Diptera Scand. 9: 3395; Walker, 1856, Insecta Britannica, Diptera 3: 140; Kertész, 1902, Catalogus Dipterorum, 1: 128; Enderlein, 1912, Zool. Anz., 40: 263; 1926, Zool. Anz., 68: 137; 1936, Die Tierwelt Mitteleuropas, 6, Ins. 3, p. 54; Melander, 1916, State Coll. Wash. Ag. Exp. Sta. Bull. 130, p. 6; Edwards, 1925, Ann. Appl. Biol., 12: 270; 1929, Dipt. of Patagonia & S. Chile, pt. 2, fasc. 3, p. 93; Duda, 1928, *in* Lindner, Die Fliegen der Palaearktischen Region, 2, fasc. 5, p. 6; Séguy, 1940, Fauna de France, 36: 296. Type species: *S. notata* (Linnaeus) by subsequent designation of Latreille (1810, Considérations Générales, p. 442, as *S. albipennis* Fab. = *S. notata* L.)

Reichertella Enderlein, 1912. Zool. Anz., 40: 268. Type species: *S. femoralis* End. (*nec.* Meigen) = *S. nigra* Mg.

Coboldia Melander, 1916. St. Coll. Wash. Ag. Exp. Sta. Bull. 130, 17–18. Type species: *Coboldia formicarum* Mel. = *S. fuscipes* Mg.

Rhaeboza Enderlein, 1936. Die Tierwelt Mitteleuropas, 6, Ins. 3, 55. Type species: *Scatopse fuscipes* Mg.

Massatierra Enderlein, 1938, *in* Skottsberg, Nat. Hist. Juan Fernandez & Easter Isl., 3, (Zoology) pp. 666–667. Type species: *Massatierra ferruginea* End. = *S. fuscipes* Mg.

[?] *Ceria* Scopoli, 1763. Entomologica Carnicolica, p. 351 [*fide* Schiner (1864) Fauna Austriaca 2: 348 and Walker (1856) Ins. Brittanica, 3: 140]. Type species: *Ceria decemnodia* Scop. = *S. notata* L.

GENERIC DESCRIPTION

The specimens of the species available to me are all rather large scatopsids ranging in length from 1.34 to 3.22 mm. in the males and 1.72 to 3.80 mm. in the females. The majority of the species exceed 2.00 mm. in total length. The wings in all species, except in *S. fuscipes*, exceed the body length. The color is generally light to dark brownish black. Several species show considerable areas of yellow. All except for *S. fuscipes* are more or less shining. The wings are clear and shining in all species, and the halteres are sordid white, yellowish or grey-brown. The legs are bicolored (yellow and brown) in some species.

Head: This is structually like that of *Swammerdamella* and *Colobostema* which have already been described and figured (1956b, fig. 6H and 1956c, fig. 2J). There are three ocelli. The eyes are holoptic in both sexes and beset with small setae. The eyes occupy the anterior one-half of the head capsule. The antennae are 10-segmented in all species and structurally like those already described. The head capsule is convex posterior to the eyes and bears scattered setae. The tentorium is complete and arises posteriorly in the premaxillary suture.

The mouth parts are as in all previously described genera, except the labellae are very large (in all but *S. fuscipes*), and the rostrum is short. The maxillary palpi are somewhat variable, but mostly short and ovate, bearing scattered setae and numerous, shallow, apparently sensory pits. The cardo-stipites are paired setaceous sclerites rather than the single fused sclerite of *Colobostema*.

Thorax: The thorax is well sclerotized and

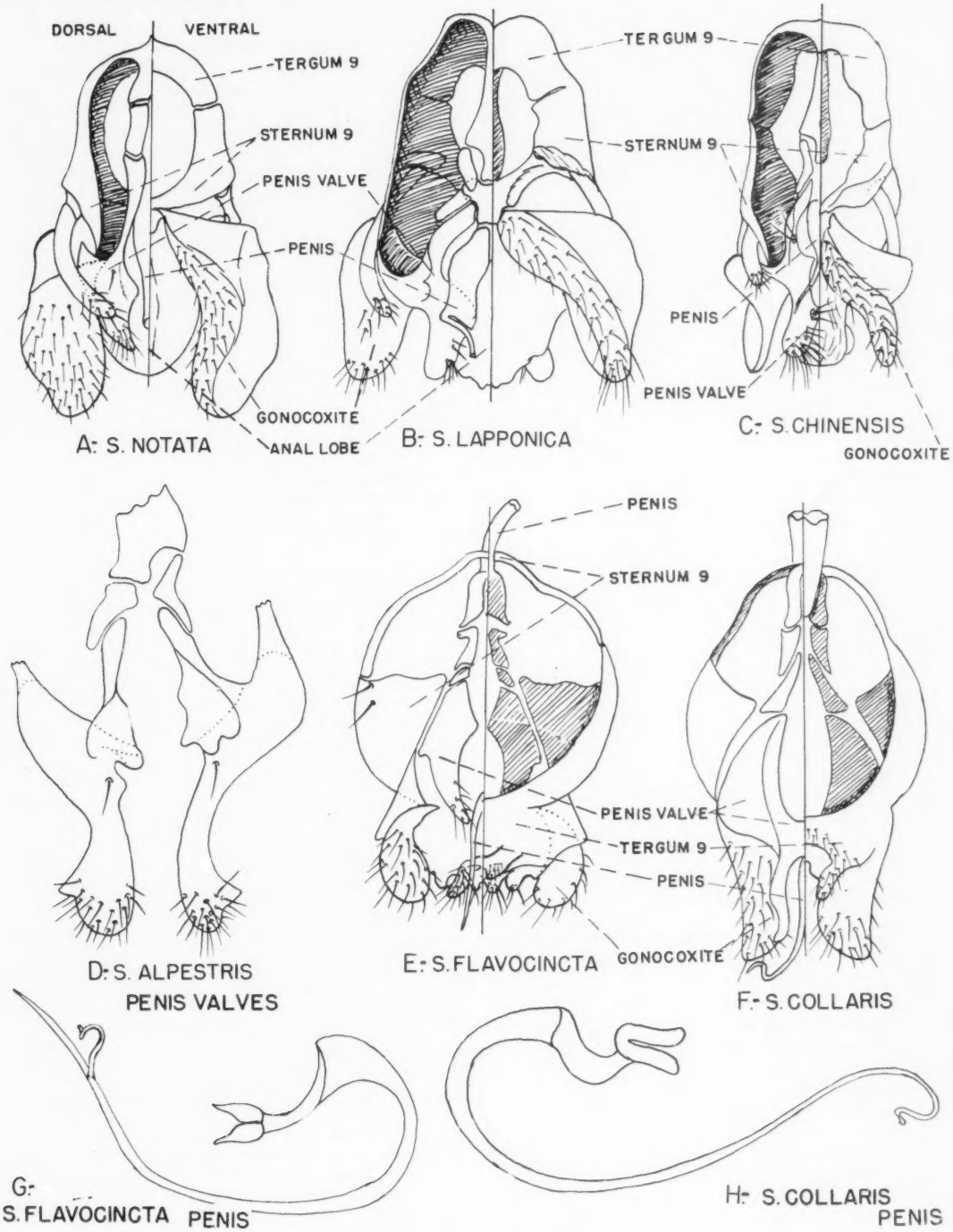

FIG. 1. A-H, Male genitalia of *Scatopse spp.*

laterally compressed. Structurally it is like that of *Swammerdamella* (1956b, fig. 6F). The anterior spiracular sclerite is small in most cases, only slightly exceeding the spiracle itself in size (fig. 3C, G-M). Only that of *S. fuscipes* (fig. 3D) approaches the large triangular shape of the sclerite in *Swammerdamella*. The supra-alar setae are well defined in the *Notata* and *Fuscipes* groups but rather poorly differentiated in most of the *Collaris* group. Subspiracular setae are rather numerous. The scutum and scutellum bear sparsely but evenly distributed setae as do the upper edge of the anepisternum and the upper half of the epimeron. There is a distinct row of setae on a separate sclerite anterior to the wing and posterior to the anepisternum. The postnotal phragma is long, extending into the second abdominal segment.

The wings (fig. 6B-F) have macrosetae on the costa, R_1 and R_3 only. The whole surface is beset with microtrichia. Section 2 of the costal margin of the wing is variable in length but usually long. With the exceptions of *S. fusipes* and *S. montana*, it is as long as or longer than Section 3. The branching veins M_1 and M_2 are long, always exceeding the basal part in length. Vein Cu_1 is nearly straight, and Cu_2 is only slightly bowed or curved except in *S. fuscipes* (fig. 6B). In the *Notata* group there is a small spur near the base of M_1 which is directed anteriorly. This spur almost attains R_3.

The halter bears a row of several setae on the pedicel.

There are no notable features in the legs that are different from any of the scatopsids considered so far. The coxae and femora are sparingly beset with short setae, the tibiae and tarsi are generally more densely setaceous. In the males of the *Notata* group the first segment of the metathoracic tarsus is either much shorter than or subequal to the second. This segment also bears a varying number of short stout spines (fig. 2G, H). The remaining species have the first segment longer than the second as in other members of the family. The claws are large and simple, and there is a broad, membraneous and setaceous empodium.

Abdomen: There are seven obvious pregenital segments. The terga and sterna are large and conspicuous, except for sternum 1 which is not developed in either sex. Tergum 1 is divided into two small, paired anterior tergites and a large, posterior, median tergite.

The seventh segment is variously modified and offers useful key characters (figs. 5C, E-J, 6A). There are seven visible pairs of spiracles in the males and eight in the females. Microsetae are very sparse on the terga and sterna except in *S. nigra*, *S. fuscipes* and *S. montana*. This results in the glossy appearance of the majority of the species and the dull appearance of the three species named above. The abdominal membrane is typically ribbed and folded.

Male Genitalia: The genitalia in this group are large and highly modified (figs. 1A-H, 2A-F, I, J, 3A, B and E, F). All are rotated through 180° and are illustrated in their normal position.

In the *Notata* group (fig. 1A-D) there is a pair of large, setaceous appendages arising ventrally which are assumed to be gonocoxites. On each side of the heavily sclerotized penis there is a pair of variably shaped penis valves. These articulate to the base of the penis through a single pair of small sclerites. There is in this group a pair of finger-like appendages which articulate to the ventral surface of segment 9. The homology of these is obscure. The anal opening is on a median lobe between the gonocoxites and topographically ventrad of the penis. In the *Collaris* group the gonocoxites are either present, reduced, or absent (figs. 1E, F, 2A-F, I, J). The penis valves are also highly modified. They may be fused together or distinct, or enormously enlarged. There are no additional appendages apparent. The most aberrant structure is present in *S. producta* (fig. 2F). The homologies in this species are quite obscure. In the *Fuscipes* group (fig. 3A, B and E, F), there is either one pair of appendages or none. In *S. nigra* (fig. 3E, F) there are no appendages except for a small pair of cerci surrounding the anus. In *S. fuscipes* there is a single pair of appendages which, although displaced posteriorly, probably are the penis valves. The genitalia of *S. brevipalpis* (fig. 2D, E) are similar in part to those of the *Fuscipes* group, but they have obvious penis valves as in the *Notata* and *Collaris* groups. *S. montana* (fig. 2I) resembles *S. brevipalpis* in the structure of the penis valves.

There is a large, free vesica present in all species.

Female Genitalia: The genitalia offer excellent specific characters as in most of the preceding genera. In the *Notata* group (fig. 4A-D) tergum 8 is conspicuous although deeply emarginate anteriorly and posteriorly, and the cerci are rather small. Ventrally *S. notata* bears a pair of posteriorly directed processes arising from sternum 8. These processes are absent from the other members of the group.

In the *Collaris* group (fig. 4E-H) the cerci are much larger than in the *Notata* group, and while tergum 8 is similar, it is usually secondarily divided into an anteromedian sclerite and a pair of posterolateral sclerites. All species (except *S. producta*) bear a pair of posteriorly directed processes on sternum 8 and sternum 9. These occupy the position of the ovipositor valves of more primitive insects. In *S. producta* (fig. 4H) only a single pair of small, posteriorly directed processes is apparent which seem to arise from sternum 9. *S. fuscipes* also has a single pair of processes on the sternal area (fig. 5D). *S.*

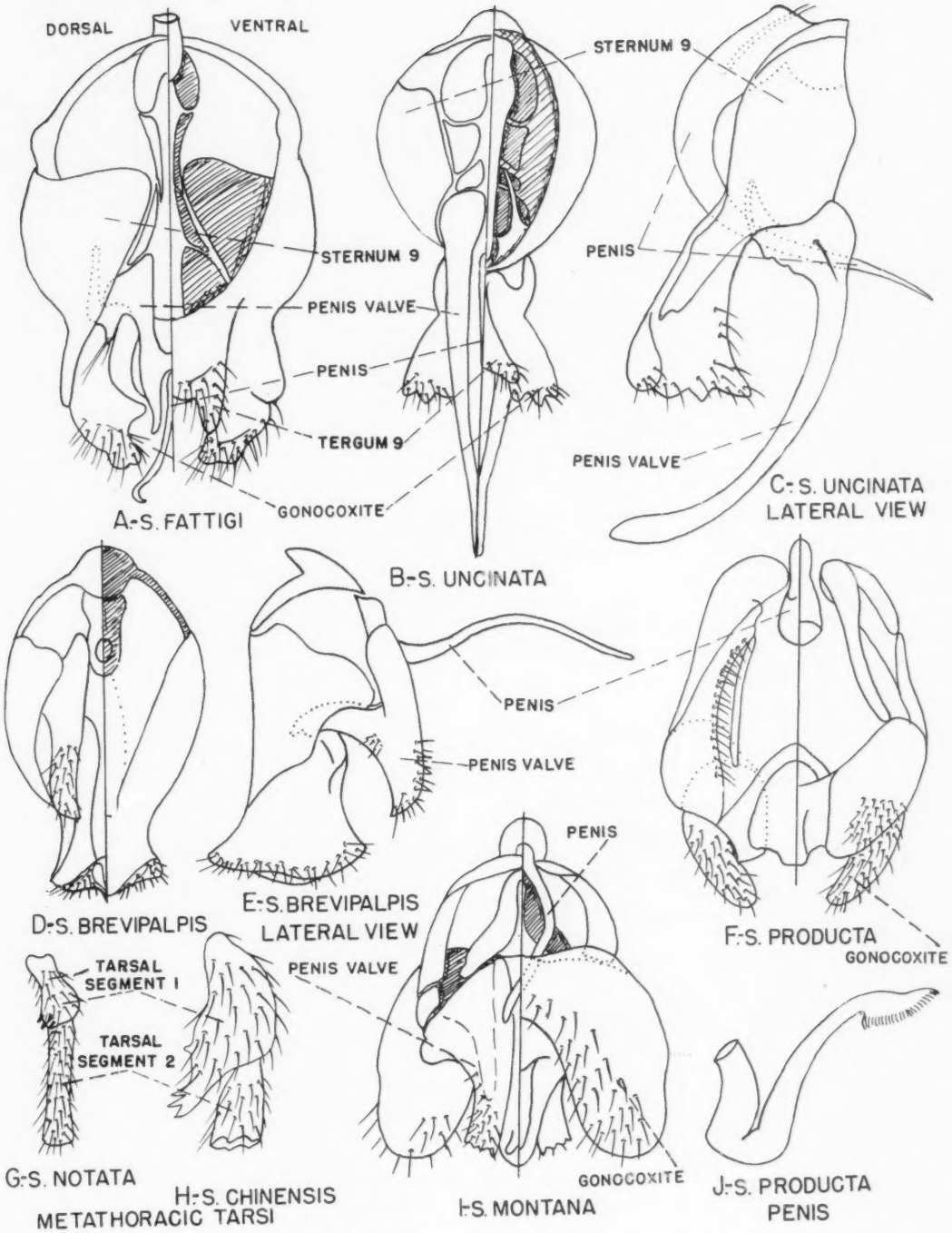

FIG. 2. A-F, I, J, Male genitalia of *Scatopse spp.*; G, metathoracic tarsi of male *S. notata*; H, metathoracic tarsi of male *S. chinensis*.

montana (fig. 5A) and *S. brevipalpis* (fig. 5B) are quite aberrant and their genitalia resemble those of *Rhexoza* more than those of other species of *Scatopse*.

A single spermatheca is present in all species.

KEY TO THE MALES OF THE SPECIES OF *SCATOPSE* KNOWN FROM NORTH AMERICA

1. Segment 1 of metathoracic tarsi 0.5 to 1.10 times as long as segment 2; vien M_1 with a short, anteriorly directed spur near base (fig. 6E) 2
1a. Segment 1 of metathoracic tarsi 1.50 to 2.60 times as long as segment 2; vein M_1 with no spur near base 4
2. Penis valves distally enlarged and setaceous (fig. 1D); R_3 with a single row of setae ventrally ..... **alpestris**
2a. Penis valves not distally enlarged; R_3 with one or more rows of setae ventrally 3
3. Gonocoxites projecting well beyond penis valves; penis valves claw-like distally (fig. 1A); R_3 with three to five rows of setae ventrally ..... **notata**
3a. Gonocoxites not projecting beyond penis valves; penis valves apically rounded (fig. 1B); R_3 with only a single row of setae ventrally ..... **lapponica**
4. Section 2 of costal margin shorter than section 3 ... 5
4a Section 2 of costal margin longer than section 3 ... 7
5. Tergum 7 produced medially in a posteriorly directed, truncate lobe (fig. 5F, G) 6
5a. Tergum 7 not posteriorly produced in a truncate lobe; genitalia as in fig. 2I ..... **montana**
6. Penis forming an open spiral, a pair of cercus-like lobes at distal end of genitalia (fig. 3A, B); sternum 7 as in fig. 5F; body dull ..... **fuscipes**
6a. Penis terminating in a brush-like structure (fig. 2J), not in the form of a spiral, cercus-like lobes directed posteriorly (fig. 2F); sternum 7 as in fig. 5G; body shining ..... **producta**
7. Penis valves produced into long, apically fused, black hooks (fig. 2B, C) ..... **uncinata**
7a. Penis valves not so produced 8
8. Tergum 7 posteriorly produced in a rounded lobe, sternum with a narrow median incision (fig. 6A) ..... **brevipalpis**
8a. Tergum 7 not produced posteriorly, sternum 7 may be broadly emarginate posteriorly 9
9. Tergum 7 with posterior margin straight; distributed west of Rocky Mts ..... **collaris**
9a. Tergum 7 smoothly rounded posteriorly (fig. 5H); distributed east of the Great Plains ..... **fattigi**

KEY TO THE FEMALES OF *SCATOPSE* KNOWN FROM NORTH AMERICA

1. Vein M_1 with a short anteriorly directed spur at base (fig. 6E) 2
1a. Vein M_1 without any trace of an anterior spur 4
2. Sternum 8 with a pair of posteriorly directed appendages (fig. 4A) ..... **notata**
2a. No appendages on sternum 8 3
3. Tergum 8 posteriorly prolonged laterally, almost enveloping cerci; lateral lobes of sternum 8 large (fig. 4C) ..... **alpestris**
3a. Tergum 8 posteriorly emarginate but not enveloping cerci; lateral lobes of sternum 8 small (fig. 4D) ..... **lapponica**
4. Genitalia with two pairs of posteriorly directed appendages on ventral surface, one pair from sternum 8, one pair from sternum 9 5
4a. Genitalia with a single pair of appendages or appendage-like lobes, or none on ventral surface 7
5. Appendages of sternum 9 broad, triangular (fig. 4G) ..... **uncinata**
5a. Appendages of sternum 9 not triangular 6
6. Appendages of sternum 8 broad and blade-like (fig. 4F) ..... **fattigi**
6a. Appendages of sternum 8 slender, finger-like (fig. 4E) ..... **collaris**
7. Sternum 8 with a pair of conspicuous, finger-like lobes projecting posteriorly (fig. 5D) ..... **fuscipes**
7a. With no trace of, or at most a vestige of a pair of lobes at posterior margin of sternum 8 8
8. With a pair of vestigial lobes on sternum 8 (?) (fig. 4H). Tergum 8 distinctly setaceous ..... **producta**
8a. With no trace of sternal lobes or appendages 9
9. Tergum 8 large with anterior and posterior margins widely separated at midline (fig. 5B) ... **brevipalpis**
9a. Tergum 8 rather small, much reduced to only a narrow strip of sclerotization at midline (fig. 5A) ..... **montana**

Scatopse notata (Linnaeus)

Tipula notata Linnaeus, 1758. Syst. Naturae, Ed. 10, p. 588, 33.

[?] *Ceria decemnodia* Scopoli, 1763. Entomologica Carnicolica, p. 351 [*fide* Walker, 1856].

Tipula latrinarium Degeer, 1776. Mem. pour servir des Ins. 6: 430.

Scatopse dubia Meigen, 1804. Klassif. Ins., p. 106.

Scatopse minuta Meigen, 1818. Syst. Besch., 1, p. 235 [*fide* Duda, 1928].

Scatopse punctata Meigen, 1818. Syst. Besch., 1, p. 235.

[?] *Scatopse vernalis* Meigen, 1818. Syst. Besch., 1, p. 235.

[?] *Scatopse fulvitarsis* Macquart, 1834. Suite à Buffon, 1, p. 181.

Scatopse femoralis Meigen, 1838. Syst. Besch., 8, p. 55 [*fide* Duda, 1928].

Scatopse glabra Meigen, 1838. Syst. Besch., 7, p. 54 [*fide* Duda, 1928].

Scatopse nitens Walker, 1848. List of Dipterous Insects in British Museum, 1, p. 114 [*fide* Paul Freeman, British Museum (Natural History), *in litt.*].

[?] *Scatopse carbonaria* Phillipi, 1865. Verh. Zool. Bot. Ges. Wien, 15: 640.

Scatopse longipennis Skuse, 1889. Proc. Linn. Soc. N. S. Wales, (2)3: 1383–4.

Scatopse notata (Linn.), Kertész, 1902, Cat. Dipt., I, pp. 131–2 (complete bibliography); Enderlein, 1912, Zool. Anz., 40: 267; Melander, 1916, State Coll. Wash. Agr. Exp. Sta. Bull. 130, p. 7; Edwards, 1925, Ann. Appl. Biol., 12: 272–4; Duda, 1928, *in* Lindner, Die Fliegen der Palaearktischen Region, 2, fasc. 5, pp. 32–3; Edwards, 1929, Dipt. Patagonia and S. Chile, pt. 2, fasc. 3, p. 94; Séguy, 1940, Fauna de France, 36: 296.

Males: Total length 2.10 to 3.10 mm. Color very dark grey-brown to jet black, shining. Setae yellow brown except supra-alars, scutellars, subspiraculars, and pedicelars black. Head jet black; antennae dull black with silvery pubescence; scutum with a bright yellow or yellow-brown spot above each wing base; membranes of pleural thoracic area yellowish, lateral membrane of abdominal segment one yellow or yellow-brown. Halter grey-brown; legs with femora, tibiae and tarsi brown, lighter than scutum or abdomen. Maxillary palpi 0.13 to 0.17 mm. long; labellae large. Enlarged supra-alar setae 15 to 30; subspiraculars 10 to 24; setae on pedicel of halter 4 to 10. First segment of metathoracic tarsus 0.57–0.70 (M = 0.64) times as long as second, stouter than second (fig. 2G). Anterior spiracular sclerite as in fig. 3C.

Wings clear, 2.70–3.40 mm. long M_1 with a short, anteriorly directed spur near base (fig. 6E); section 2 of costal margin 1.25–1.52 times as long as section 3; M 0.52–0.65 times as long as

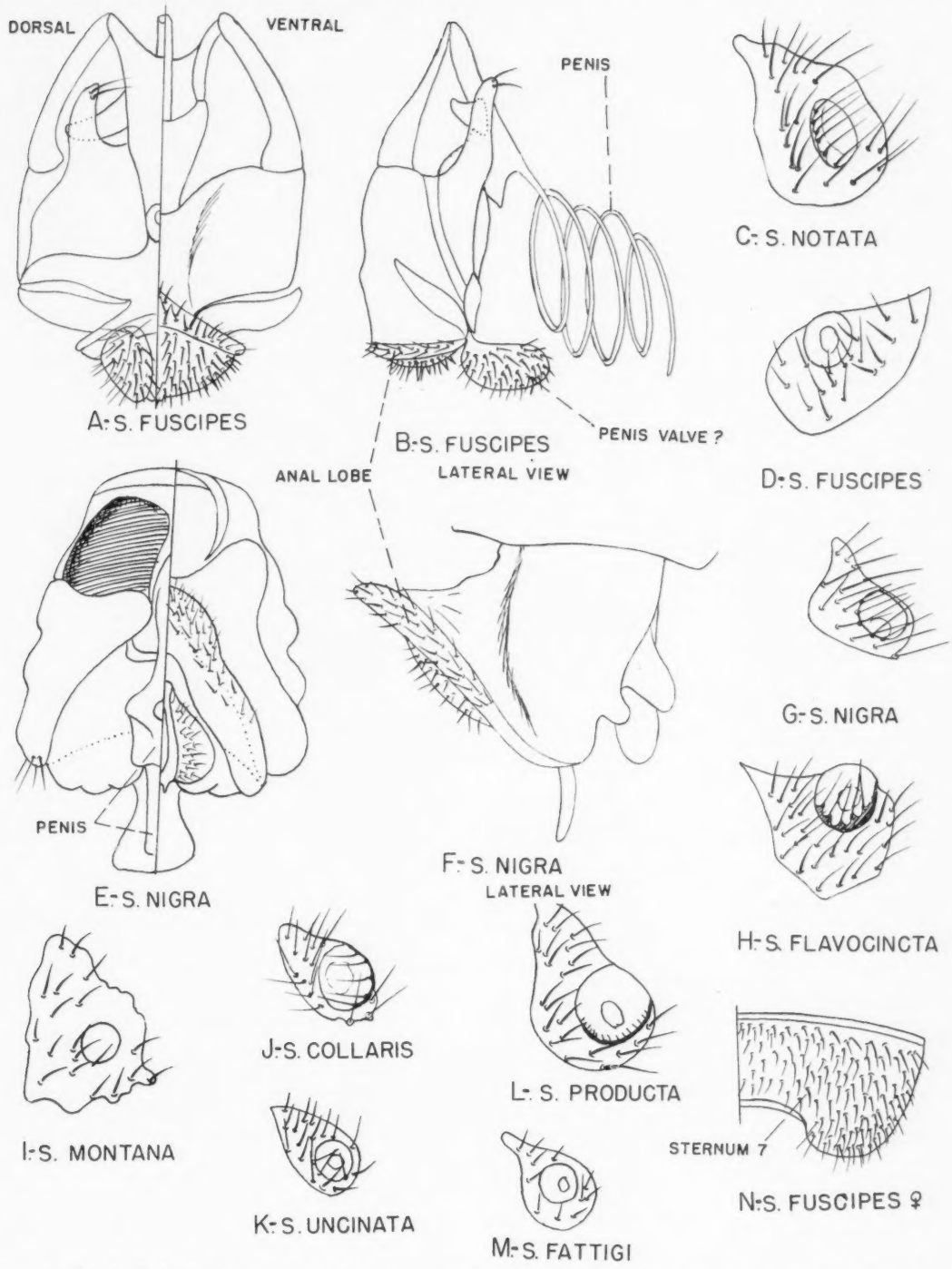

FIG. 3. A, B, E, F, Male genitalia of *Scatopse spp.*; C, D, G-M, spiracular sclerite of *Scatopse spp.*; N, sternum 7 of ♀ *S. fuscipes*.

M_2; Costa and Radius with setae; R_3 with 2 or 3 irregular rows dorsally and 3 to 5 rows of setae ventrally.

Segment 7 of abdomen as in fig. 5C. Genital vesica and apodemes 0.37 to 0.47 mm. long. Genitalia (fig. 1A) with gonocoxites large; penis valves short and stout; accessory finger-like appendages slender and digitiform.

Females: Total length 2.75–3.80 mm.; wing length 2.90–3.52 mm. Supra-alar setae 15 to 24; subspiraculars 13 to 25; pedicelars 5 to 10; 2 to 4 rows of setae on dorsal side of R_3, 3 to 4 rows on ventral side. Otherwise as male in color and chaetotaxy. Genitalia (fig. 4A) with sternum 8 produced into two posteriorly directed processes.

Comments: This cosmopolitan species is widely distributed throughout the world. Next to *S. fuscipes* it is probably the commonest in collections. It is recorded as being reared from human excrement and from other decaying organic matter. It is probable that the *Reichertella femoralis* recorded by Melander (1916) is this species. The larva of this species is known and has been described (Lyall, Ann. Appl. Biol., 16: 630–6). This species is closely related to the three immediately following species: *S. lapponica* Duda, *S. alpestris* n. sp. and *S. chinensis* n. sp.

Specimens examined: More than 1,000 specimens of this species have been available for examination from: Alabama, Alaska, Arkansas, California, Colorado, Connecticut, Georgia, Idaho, Illinois, Iowa, Indiana, Kansas, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Nevada, New Mexico, New York, Ohio, Oregon, South Carolina, Texas, Utah, Virginia, and Washington in the U. S.; and from British Columbia, Manitoba, Ontario, and Quebec in Canada. In addition, specimens have been available from Chile, England, Germany, Greenland, Iceland, New South Wales, Norway, New Zealand, and Tokasha in Siberia. It is doubtless distributed throughout Europe and North America and has apparently been carried by commerce to Australia, New Zealand and South America.

Scatopse lapponica Duda

Scatopse lapponica Duda, 1928, *in* Lindner, Die Fliegen der Palaearktischen Region, Bd. II, fasc. 5, pp. 27–28.

Males: Total length 1.86–2.00 mm. Color very dark grey brown to jet black, shining; setae yellow-brown except supra-alars, subspiraculars, scutellars and pedicelars black. Head jet black; antennae dull black with silvery pubescence; scutum with a bright yellow spot above each wing base; membranes of pleural thoracic area and abdominal sternite 1 yellow; halteres grey-brown; legs concolorous with thorax and abdomen. Maxillary palpi 0.12–0.17 mm. long. Enlarged supra-alar setae 12 to 16; subspiraculars 6 to 13; pedicelars 4 to 6. First segment of metathoracic tarsus 0.78–1.10 (M=0.91) times as long as second.

Wings 2.00–2.50 mm. long; M_1 with a short, anteriorly directed spur near base; section 2 of costal margin 0.81–0.93 times as long as section 3; M 0.57–0.81 times as long as M_2. Costa and Radius with setae; R_3 with 1 or 2 rows of setae dorsally and a single row ventrally.

Segment 7 of abdomen as in *S. notata*. Genital vesica and apodemes 0.25–0.30 mm. long.

Genitalia (fig. 1B) with gonocoxites not projecting beyond penis valves; penis valves with few setae; accessory appendages as in *S. notata* but shorter.

Females: Total length 2.06–2.96 mm.; wing length 2.20–2.80 mm. Supra-alar setae 15 to 18; subspiraculars 9 to 16; pedicelars 5 to 7; 1 or 2 rows of setae on dorsal side of R_3 and 1 (or rarely 2) on ventral side. Otherwise as male in color and chaetotaxy. Genitalia (fig. 4D) with no posterior processes on sternum 8.

Comments: This species is very close to *S. notata* as examination of the genitalia of the males as well as the wing venation reveals. It differs from *S. notata* in the smaller size, the lack of appendages on the female genitalia, the proportion of parts in the male genitalia and the chaetotaxy of vein R_3.

Duda described this species from northern Europe.

Specimens examined: SWEDEN: 6♂♂, 3♀♀, Abisko, Lapland, July 2-Aug. 12, 1951; LABRADOR: 3♂♂, 7♀♀, Hebron, July 10–19, 1954; 8♂♂, 7♀♀, July 16, 1954 (ex. flowers of crassulaceous plant); 2♂♂, 2♀♀, Aug. 5–11; 1♀, Okak Bay, July 23, 1954; MANITOBA: 1♀, Fort Churchill, July 7, 1952; 3♂♂, 2♀♀, Churchill, July 26-Aug. 11, 1952; 1♀, July 26, 1947; 1♀, Eastern Ck., Nr. Churchill, July 9, 1952; NORTH WEST TERRITORIES: 5♂♂, 10♀♀, Reindeer Depot, Mackenzie Delta, June 30-July 8, 1948; 1♀, Chesterfield, Aug. 17, 1950; QUEBEC: 1♂, 4♀♀, G. Whale River, July 8-July 30, 1949; 1♀, Indian House Lake, July 23, 1954; YUKON TERRITORY: 2♀♀, Whitehorse, June 30, July 25, 1948–9; 1♀, Dawson, June 16, 1949; 1♀, Rampart House, June 3, 1951.

Scatopse alpestris n. sp.

Males: Total length 2.41–2.92 mm. Color identical with *S. notata* but legs concolorous with rest of body. Maxillary palpi 0.13–0.15 mm. long. Enlarged supra-alar setae 17 to 23; subspiraculars 13 to 21; setae on pedicel of halter 4 or 5. First tarsal segment of metathoracic leg 0.85–1.00 times as long as second.

Wings 3.10–3.41 mm. long (M=3.26). M_1 with a short, anteriorly directed spur near base. Section 2 of costal margin 1.16–1.25 times as long as section 3; M 0.50–0.66 times as long as M_2. Costa and Radius with setae; R_3 with 2 or 3

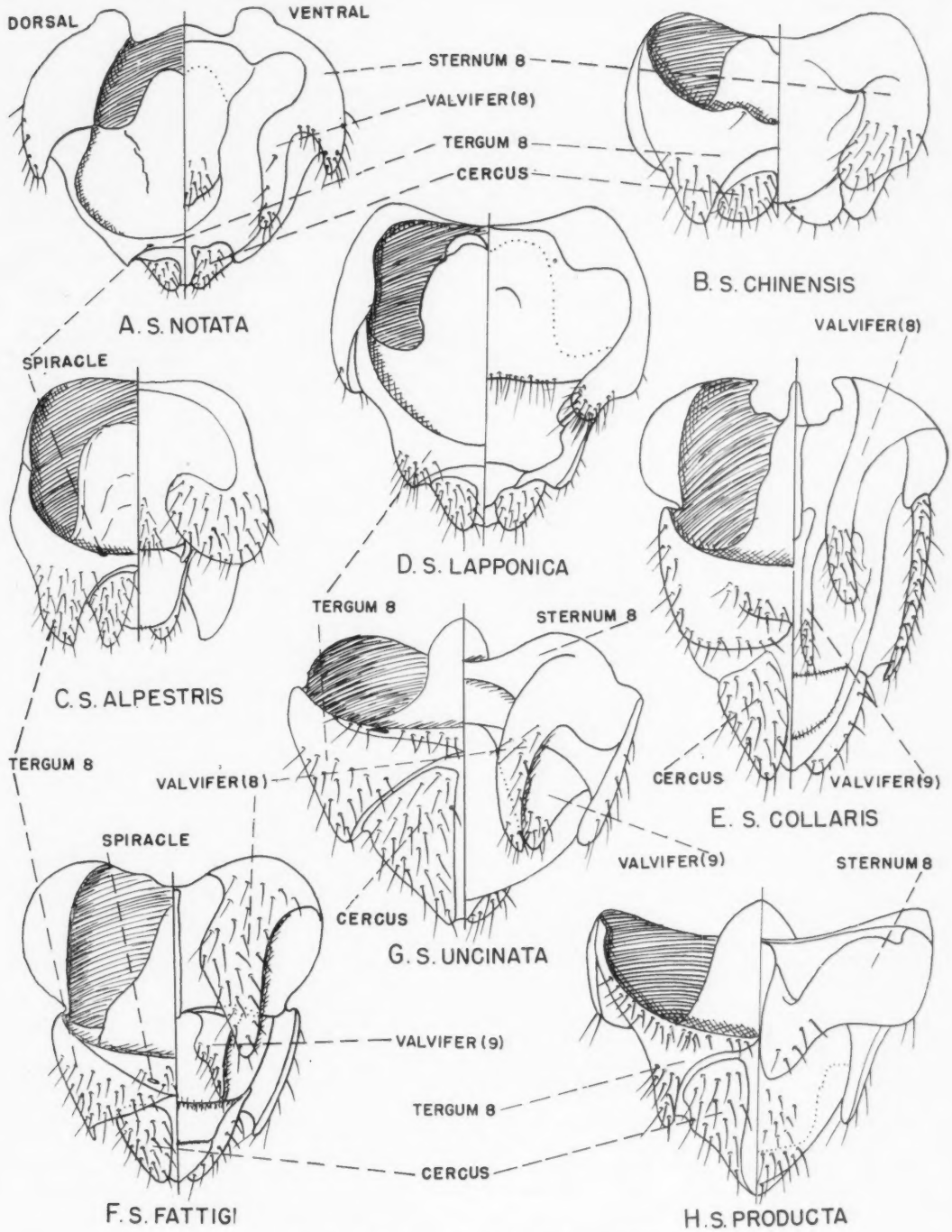

FIG. 4. A-H, Female genitalia of *Scatopse spp.*

irregular rows dorsally and a single row ventrally, as in *S. lapponica*.

Segment 7 of abdomen as in *S. notata*. Genital vesica and apodemes 0.29–0.34 mm. long.

Genitalia (fig. 1D) like those of *S. notata* with gonocoxite not exceeding penis valves in length; penis valves long and apically enlarged; accessory appendages as in *S. notata*.

Females: Total length 2.76–3.26 mm.; wing length 2.80–3.21 mm. Supra-alar setae 13 to 22; subspiraculars 8 to 15; pedicelars 9 or 10; 1–2 or 1–3 rows of setae on dorsal side of R_3 and 1–3 or 2–3 rows on ventral side. Otherwise chaetotaxy and color as in male.

Genitalia (fig. 4C) without posteriorly directed appendages on sternum 8.

Comments: In size this species approaches *S. notata*; however the lack of appendages on the female genitalia link it more closely to *S. lapponica*. In distribution it is apparently confined to the high areas of the Rocky Mts. and to Alaska.

Specimens examined: Holotype: ♂, Tennessee Pass, Colorado, July 23, 1917, J. M. Aldrich. Allotype: ♀, Tennessee Pass, Colorado, July 7, J. M. Aldrich. Types in the U. S. National Museum. Paratypes: ALASKA: 1♀, Juneau, July 25, 1899; 1♀, Rapids, July 18, 1937; 2♀♀, Ruby, June 29, 1929; 1♂, Popoff Is., July 10, 1899; 1♂, Anchorage, July 22, 1921; 1♀, July 3, 1951; 1♂, Matanuska, May 25, 1944; 1♀, Naknek, Aug. 2, 1952 (on tundra); ALBERTA: 3♀♀, Banff, June 17-July 19, 1922; BRITISH COLUMBIA: 1♂, 4♀♀, Diamond Head Chalet, Garibaldi Park (5000 ft.), Aug. 28, 1953; COLORADO: 2♂♂, 1♀, same data as allotype; 1♀, Marshall Pass, July 28, 1908, 1♀, Cameron Pass, Aug. 21, 1940; 2♀♀, Florissant, (no other data); WASHINGTON: 1♂, Mt. Rainier, Aug. 1922; WYOMING: 6♂♂, Yellowstone Natl. Park, Aug. 9–12, 1918.

Scatopse chinensis n. sp.

Males: Total length 1.90 mm.; color very dark grey-brown; very like *S. notata* except in the two specimens available the pale areas of the thorax and abdomen are sordid white rather than yellow, and the legs are concolorous with the thorax and abdomen. Maxillary palpi 0.12 mm. long. Supra-alar setae 13; subspiraculars 9; pedicelars 6. First tarsal segment of metathoracic leg 0.5 times as long as second, distinctly produced apically and armed with a row of three spines.

Wings 2.62–2.69 mm. long. M_1 with a short, anteriorly directed spur near base. Section 2 of costal margin 0.97–1.03 times as long as section 3; M 0.44–0.45 times as long as M_2; R_3 with a single row of setae both dorsally and ventrally.

Segment . of abdomen as in *S. notata*. Genital vesica and apodemes 0.30 mm. long.

Genitalia (fig. 1C) with gonocoxites small; penis valves large, stout; accessory appendages short.

Female: Total length 2.19 mm.; wing length 2.69 mm. Supra-alar setae 14; subspiraculars 16; pedicelars 6; 1 row of setae on dorsal and ventral sides of R_3. Otherwise identical with male in color and chaetotaxy, except abdominal setae more numerous and color dull.

Genitalia (fig. 4B) with only slight processes on sternum 8.

Comments: This is close to *S. notata*, but the slender, smaller male genitalia and the lack of large processes on the female genitalia serve to distinguish it from that species.

Specimens examined: Holotype: ♂, Chengtu, Szechuen, China, Feb. 16, 1924, D. C. Graham. Allotype: ♀, Suifu, Szechuen, China (no other data). Types in the U. S. National Museum. Paratype: 1♂, same data as holotype.

Scatopse collaris (Melander), new combination

Reichertella collaris Melander, 1916. State Coll. Wash. Ag. Exp. Sta. Bull. 130, p. 10.

Type locality: Chahalis, Washington.

Males: Total length 2.06–2.86 mm. Color generally dark grey-brown, somewhat shining; all setae yellowish. Head jet black, shining; antennae dull black with silvery pubescence; scutum dark grey-brown; scutellum yellow to yellow-brown; pleural areas lighter, shining brown; prothorax yellow to yellow-brown; anterior spiracular sclerite yellow to yellow-brown; membranes whitish. Prothoracic coxae yellow to yellow-brown; remaining coxae darker brown; legs otherwise paler yellow to yellow-brown except apical third of tibiae darker. Wings with a light brownish cast; halteres pale yellowish to yellow-brown. Abdomen with dark grey-brown, shining terga and sterna; membrane of segments 1 to 3 pale yellow or grayish yellow.

Maxillary palpi 0.08–0.12 mm. long, ovoid; labellae large and rostrum short, less than one-third total head height (including rostrum). Supra-alar setae not especially differentiated, yellow in color, 10 to 18 present; subspiraculars 2 to 11; pedicelars 6 to 8; first tarsal segment of metathoracic leg 1.44–1.75 times as long as second. Spiracular sclerite as in fig. 3J.

Wings (fig. 6F) 2.69–3.25 mm. long; section 2 of costal margin 1.40–1.58 times as long as section 3; M 0.53–0.71 times as long as M_2. R_3 with a single row of setae dorsally and ventrally.

Sternum 7 deeply incised posteriorly (fig. 5J); tergum 7 with a straight posterior margin. Genital vesica and apodemes 0.46–0.56 mm. long.

Genitalia (fig. 1E) with penis valves small; gonocoxites with margins entire; penis elongate terminating in a short spiral (fig. 1H).

Females: Total length 2.24–3.07 mm.; wing length 2.83–3.52 mm. Otherwise as males in color and chaetotaxy.

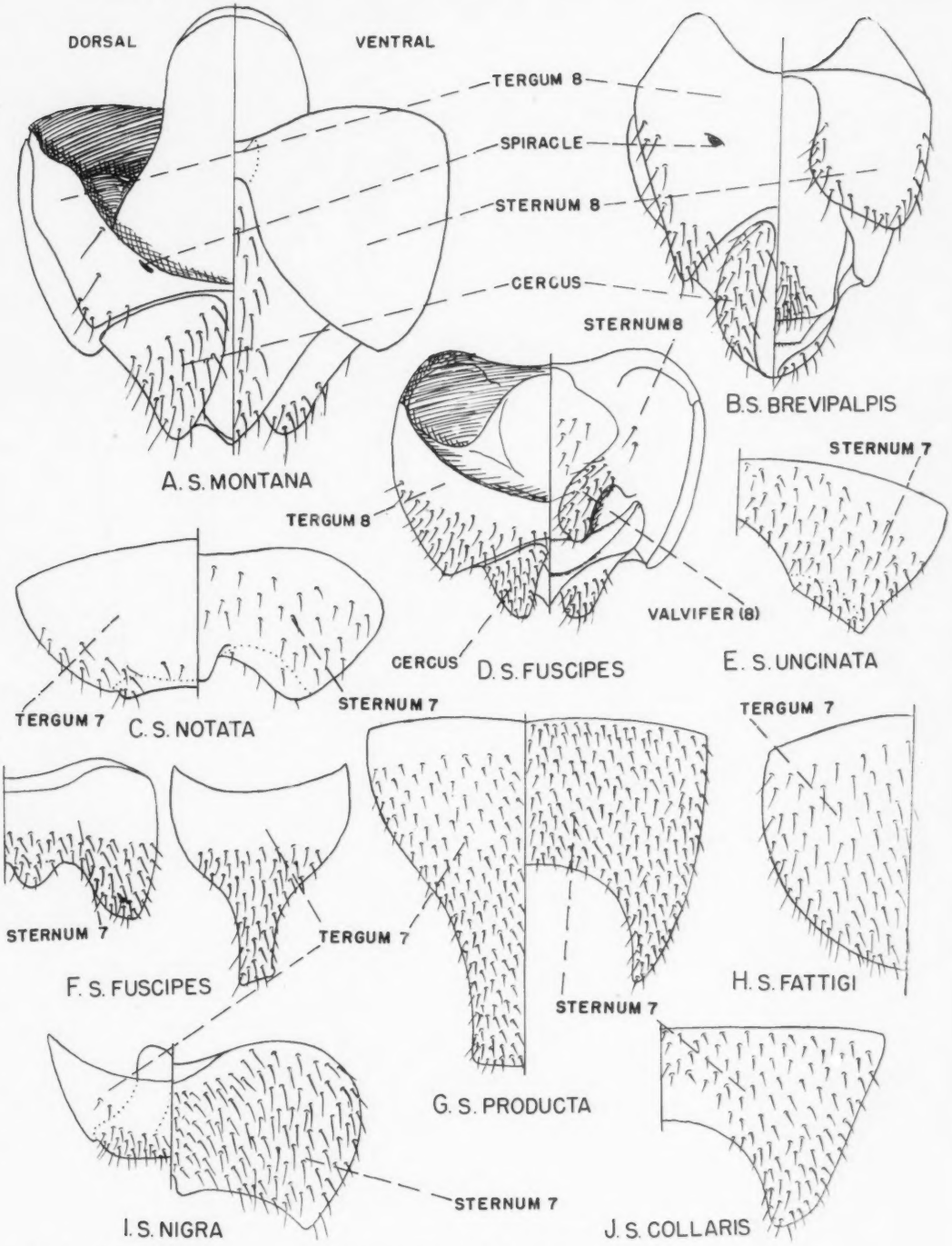

FIG. 5. A, B, D, Female genitalia of *Scatopse spp.*; C, E-J, sclerites of segment 7 of males of *Scatopse spp.*

Genitalia (fig. 4E) with a pair of slender appendages on posterior margins of sterna 8 and 9. Cerci large; tergum 8 medially incised posteriorly.

Comments: This species is distributed along the Pacific Coast from central California to British Columbia. It is apparently generally found at lower elevations than *S. uncinata*. It is distinguished readily from *S. uncinata* by the yellow or yellow-brown pronotal lobes and the pale scutellum. In addition, the males lack the long hook-like penis valves, and the appendages of sterna 8 and 9 in the females are slender and not blade-like.

Specimens examined: British Columbia: 1♂, Victoria, Sept. 21, 1916; California: 3♂♂, Corte de Madera Creek, nr. Portola; 14♂♂, 25♀♀, Los Gatos, Nov. 13, 1943; 7♂♂, 3♀♀, Berkeley, Oct. 31, 1947; Idaho: 1♂, 1♀, Chatcolet, Aug. 1915; 1♂, 2♀♀, Priest Lake, Sept. 1–3, 1919; 1♂, Aug. 18, 1919; 1♂, 1♀, Moscow Mt., Aug. 28, 1916; 1♀, Sept. 16, 1917; 1♀, Sept. 10, 1918; 3♀♀, Sept. 10, 1922; 1♂, Aug. 26, 1923; Washington: 52♂♂, 5♀♀, Pullman, Sept. 14, 1924; 1♀, Oct, 1940; 1♂, 3♀♀, Colfax, Oct. 5, 1941; 1♂, Mt. Rainier, Aug. 19, 1934.

Scatopse uncinata (Melander), new combination

Reichertella uncinata Melander, 1916. State Coll. Wash. Ag. Exp. Sta. Bull. 130, pp. 10–11.

Type locality: Douglas, Alaska.

Males: Total length 1.69–2.28 mm. Color dark reddish-brown with considerable yellow, shining; setae all pale. Head jet black, shining; antennae dull black with silvery pubescence; thoracic sclerites dark reddish-brown, scutum darkest; membranes of thorax and anterior spiracular sclerite yellow or sordid white; halteres yellow or whitish; legs concolorous with scutum except basal half of tibiae yellow; wings with a light brown cast; membrane between terga and sterna of segments 1 to 3 yellow or sordid white; remainder of abdomen brown, shining.

Maxillary palpi 0.08–0.10 mm. long, ovoid; labellae large and rostrum short, less than one-fourth of head height (inc. rostrum). Supra-alar setae not well differentiated, 10–12 present; subspiraculars 5 to 12; pedicelars 4 to 6. First tarsal segment of metathoracic leg from 1.66–2.00 times as long as second. Spiracular sclerite as in fig. 3K.

Wings 2.66–3.44 mm. long; section 2 of costal margin 1.36–1.52 times as long as section 3; M 0.51–0.66 times as long as M_2; R_3 with a single row of setae both dorsally and ventrally.

Sternum 7 of abdomen as in fig. 5E. Genital vesica and apodemes 0.43 mm. long.

Genitalia (fig. 2B, C) with penis valves enormously enlarged forming a pair of black, downcurved, apically approximated hooks on either side of penis; gonocoxites fused basally.

Females: Total length 1.86–3.10 mm.; wing length 2.51–3.45 mm. Supra-alar setae 8 or 9; subspiraculars 6 or 7; pedicelars 6 or 7. Otherwise as male in color and chaetotaxy.

Genitalia (fig. 4G) with sterna of both segments 8 and 9 produced into flat, blade-like appendages (ovipositors); cerci large; small spiracles present on tergum 8.

Comments: This species ranges from Wyoming and Washington north to the Alaskan peninsula. In the males it is readily recognized by the black, hook-like penis valves and in the females by the blade-like ovipositors.

Specimens examined: Alaska: 1♂, 3♀♀, Cold Bay, 163° W (on Tundra), Aug. 13, 1952; British Columbia—Alberta: 2♂♂, 2♀♀, Great Divide, July 30, 1935; British Columbia: 1♂, 1♀, Mt. Harry, Mt. Revelstoke National Pk. (6200 ft.), Aug. 11, 1952; 2♂♂, 10♀♀, Eva Lake Trail, Mt. Revelstoke Pk. (6000) ft.), Aug. 6–19, 1952; 1♀, Glacier, July 27, 1916; Montana: 1♀, Glacier National Park, July 24, 1925; Washington: 5♂♂, 17♀♀, Mt. Rainier, July 24, 1924; 3♂♂, Sept. 1, 1917; 2♀♀, Aug. 1921; 1♀, Aug. 1917; 1♀, Aug. 1934; 6♂♂, 7♀♀, Mt. Baker, Aug. 10, 1925; Wyoming: 2♂♂, 2♀♀, Yellowstone National Park, July 28, 1934.

Scatopse fattigi n. sp.

Males: Total length 2.00–2.40 mm. Color generally golden brown to dark brownish-black, shining; all setae pale golden yellow; head jet black, shining; antennae dull black with silvery pubescence; scutum and dorsum of abdomen darkest; halteres pale yellowish; legs all yellow-brown; wings with a brownish cast owing to rather long, brown microtrichia.

Maxillary palpi 0.10 mm. long, ovoid; labellae large; rostrum quite short, less than one-fifth total head height (including rostrum). Supra-alar setae 12; subspiraculars 5, pedicelars 5. First tarsal segment of metathoracic leg 1.86–1.88 times as long as second. Spiracular sclerite as in fig. 3M.

Wings 2.55–3.10 mm.; section 2 of costal margin 1.60–1.67 times as long as section 3; M 0.62–0.68 times as long as M_2; R_3 with a single row of setae both dorsally and ventrally.

Segment 7 with tergum smoothly rounded posteriorly and sternum deeply emarginate (fig. 5H). Genital vesica and apodemes 0.51 mm. long.

Genitalia (fig. 2A) resembling those of *S. collaris;* penis valves somewhat shorter and gonocoxite with a large tooth on mesal face.

Females: Total length 2.06–2.41 mm.; wing length 2.76–3.00 mm. Otherwise as males in color and chaetotaxy. Genitalia (fig. 4F) with a pair of broad, blade-like appendages on posterior margins of both sterna 8 and 9, similar to those of *S. collaris;* tergum 8 divided into two lateral and one small, triangular, anteromesal

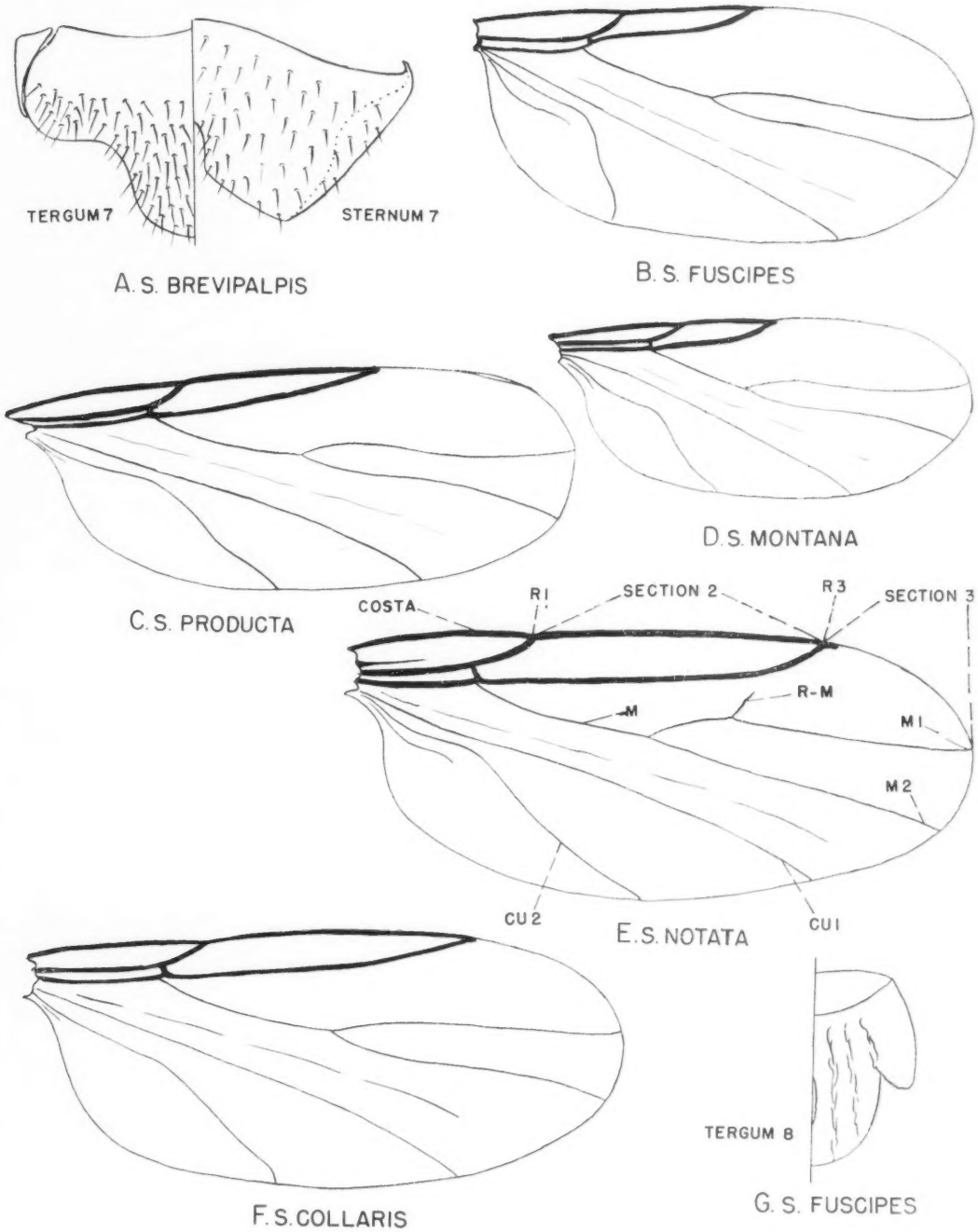

FIG. 6. A, Segment 7 of *S. brevipalpis*; B-F, Wings of *Scatopse spp.*; G, tergum 8 of male *S. fuscipes*.

sclerites; spiracles present on anteromesal piece. Cerci large.

Comments: This rare species is known from Georgia to Ontario, Quebec and West to Wisconsin. It is most closely related to the western species *S. uncinata* and *S. collaris* as evidenced by the presence of ovipositors on sterna 8 and 9 in the female and the genital structure of the males. It can be distinguished from its nearest eastern relatives *S. brevipalpis* and *S. producta* by the all yellow or yellowish-brown legs.

Specimens examined: Holotype: ♂, WASHINGTON, D. C., Oct. 5, 1894 (no other data). Allotype: ♀, Stone Mt., GEORGIA, November 21, 1943, P.W. Fattig. Types in U. S. National Museum Collection. Paratypes: 1♂, Stubblefield Falls, VIRGINIA, on *Pinus virginianus*, Oct. 30, 1921; 2♀♀, Pittsburg, PENNSYLVANIA, Sept. 22–30 and Oct. 5, 1911; 1♀, Washburn Co., WISCONSIN, Sept. 3, 1952; 1♀, Laniel, QUEBEC, Sept. 20, 1933; 1♂, Maynooth, ONTARIO, from rotting fungi, Aug. 30, 1952; 1♀, Ottawa, April 10, 1954; 1♀, Gorham, NEW HAMPSHIRE, Aug. 20, 1955.

Scatopse flavocincta Duda

Scatopse flavocincta Duda, 1928. *In* Lindner, Die Fliegen der Palaearktischen Region, Bd. 2, fasc. 5, pp. 20–21; Collin, 1954, Jour. Soc. British Ent., 5: 74–5.

Males: Total length 2.24 mm. Color very dark brownish black, shining; head jet black, shining; antennae black with yellowish-white pubescence, dull; all setae yellow-brown; halteres sordid white with pedicels pale brownish; legs concolorous with body except basal third to half of tibiae yellow-brown; wings clear, dull.

Maxillary palpi short, 0.10 mm. long, broadly oval; rostrum short, about one-third of total head height (including rostrum); labellae large. Supra-alar setae 10, not well differentiated; supspiraculars 11; pedicelars 9. First tarsal segment of metathoracic leg 1.70 times as long as second. Spiracular sclerite small relative to spiracular aperture (fig. 3H).

Wings 3.10 mm. long; section 2 of costal margin 1.92 times as long as section 3; M 0.67 times as long as M_2; R_3 with a single row of setae dorsally and ventrally.

Segment 7 of abdomen without emargination or processes. Genital vesica and apodemes 0.42 mm. long.

Genitalia (fig. 1E) with penis valves claw-like, a pair of black, lobe-like appendages apically (gonocoxites); penis large, strongly curved and apically acute, a short blunt branch near apex (fig. 1G).

Females: No females have been available, but Duda indicates no essential differences between the sexes.

Comments: The male genitalia, the structure of the rostrum and labellae and the spiracular sclerites link this species to *S. notata* and its relatives. The wing structure and wing size relative to body size is much like that of *S. uncinata* (Mel.) and *S. collaris* (Mel.) (fig. 6F).

Specimens examined: 2♂♂, Abisko, Lapland, Sweden, Aug. 12, 1951, J. R. Vockeroth.

Scatopse producta n. sp.

Males: Total length 2.14–3.22 mm. Color dark grey-brown to nearly jet black, shining; setae yellowish to grey; head jet black, shining; antennae dull black with silvery pubescence, except antennal segment 2 brown; coxae and femora concolorous with thorax; apex of femur with very narrow yellow-brown ring; basal one-third of middle and hind tibiae yellow-brown; fore tibiae mostly yellow-brown; a broad, faint, darker band on apical half; tarsi all greyish-yellow; halteres dark grey-brown; wings faintly infuscated anteriorly; abdomen all concolorous with thorax.

Maxillary palpi 0.12–0.13 mm. long, ovoid; labellae large; rostrum short, less than one-third of total head height (including rostrum). Enlarged supra-alar setae 15 or 16; subspiraculars 8 to 13; pedicelars 6 or 7. First tarsal segment of metathoracic leg 1.60–1.90 times as long as second. Spiracular sclerite as in fig. 3L.

Wings (fig. 6C) 2.76–3.10 mm. long; second section of costal margin 0.87–0.93 times as long as section 3; M 0.50–0.55 times as long as M_2; R_3 with a single row of setae dorsally only.

Segment 7 of abdomen (fig. 5G) produced posteriorly in a broad, truncate lobe; sternum broadly emarginate posteriorly. Genital vesica and apodemes 0.29–0.36 mm. long.

Genitalia (fig. 2F) with gonocoxites small and rounded; penis valves reduced; penis apically enlarged, beset with spines (fig. 2J).

Female: Total length 2.76 mm.; wing length 2.50 mm.; otherwise as male.

Genitalia (fig. 4H) without any appendages except for small, approximated lobes on sternum 8. Tergum 8 very narrow medially; cerci large.

Comments: This species is readily distinguished in the males from those preceding by the production of tergum 7 into a broad, truncate process posteriorly. In the females the bicolored tibiae distinguish it from all eastern species except *S. brevipalpis*. It can be distinguished from the latter by the presence of setae on the dorsal side of R_3 only and by the fact that the second section of the costa is subequal to the third rather than longer than the third.

Specimens examined: Holotype: ♂, Lost River State Park, WEST VIRGINIA, (Spruce, 3200 ft.), May 23, 1954. W. W. Wirth. Allotype: ♀, same data as holotype. Types in the U. S. National Museum Collections. Paratypes: 1♂, same data as holotype; 2♂♂, Smokey Mts., TENNESSEE, alt. 5800 ft., June 6, 1939; 1♂, Beverly, MASSACHUSETTS, June 6, 1915.

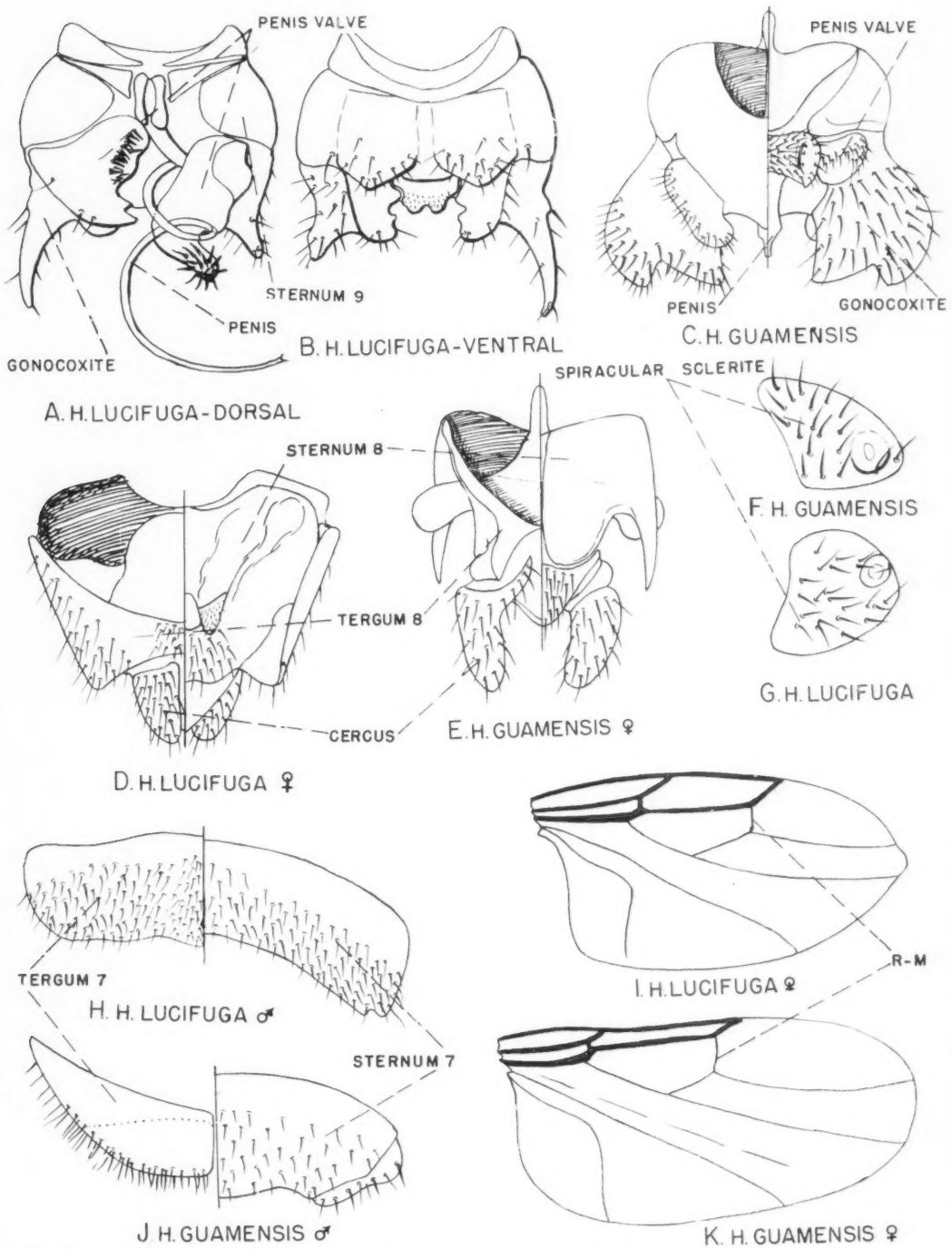

FIG. 7. A-C, Male genitalia of *Holoplagia* spp.; D, E, female genitalia of *Holoplagia* spp.; F, G, spiracular sclerite of *Holoplagia* spp.; H, J, segment 7 of males of *Holoplagia* spp.; I, K, wings of female *Holoplagia* spp.

Scatopse montana n. sp.

Males: Total length 1.55–1.93 mm. Color very dark grey-brown, somewhat shining; head jet black; antennae dull black with silvery pubescence; setae largely pale; halteres very dark grey; legs concolorous with body. Rostrum less than one-third total head height (including rostrum); labellae large.

Maxillary palpi elongate oval, 0.12 mm. long. Enlarged supra-alar setae 13 or 14; subspiraculars 4 to 7; pedicelars 5 or 6. First tarsal segment of metathoracic leg 1.70–1.83 times as long as second. Anterior spiracular sclerite small, as in fig. 3I. Wings (fig. 6D) clear with M and Cu clearly apparent even in slide mounts; length 1.58–1.96 mm.; section 2 of costal margin 0.43–0.68 times as long as section 3; M 0.45–0.58 times as long as M_2; R_3 with a single row of setae on dorsal side only.

Segment 7 of abdomen rather asymmetrical; tergum broadly produced, sternum deeply emarginate. Genital vesica and apodemes 0.27–0.30 mm. long.

Genitalia (fig. 2I) with a medial pair of appendages (penis valves) and a pair of large, fused, lobe-like structures ventrally (gonocoxites). Penis large basally, terminating in a slender loop.

Females: Total length 2.30–2.69 mm.; wing length 1.90–2.24 mm. Supra-alar setae 12; subspiraculars 6; pedicelars 6; R_3 with a single row of setae on dorsal side only. Otherwise as males in color and chaetotaxy.

Genitalia (fig. 5A) without appendages on sternum 8; sternum 8 deeply emarginate posteriorly; tergum 8 emarginate anteriorly and posteriorly, very slender medially but with a pair of small spiracles; cerci large.

Comments: The male genitalia in this species show a closer relationship to *S. notata* and the *Collaris* group than to those of the *Fuscipes* group.

Specimens examined: Holotype: ♂, Glacier Park, Logan Pass, MONTANA, July 18, 1935, A. L. Melander. Allotype: ♀, same data as holotype. Types in the collection of A. L. Melander. Paratypes: 6♂♂, 3♀♀, same data as holotype; 1♂, Mt. Rainier, Eagle Peak, WASHINGTON, July 19, 1922; 1♂, Mt. Adams, Washington, July 24, 1921.

Scatopse brevipalpis n. sp.

Male: Total length 2.06 mm. Color generally dark brownish-black, shining; head jet black, feebly shining; antennae dull black; sides of thorax grey, shining; membrane of pleura yellow; abdomen dark brownish-black, feebly shining dorsally and ventrally, laterally dull. First abdominal sternum yellow; legs generally colored as body except basal half of tibiae yellow; setae generally yellowish-brown; halteres sordid white; wings clear, somewhat grey, not shining.

Maxillary palpi small, ovoid, 0.08 mm. long; rostrum short, less than one-third of total head height (including rostrum); labellae large. Enlarged supra-alar setae 6; subspiraculars 8; pedicelars 5. Anterior spiracular sclerite only a narrow rim around spiracle. First tarsal segment of metathoracic leg 1.65 times as long as second.

Wings 2.20 mm. long; section 2 of costal margin 1.32 times as long as section 3; M 0.56 times as long as M_2; R_3 with a single row of setae dorsally and ventrally. Segment 7 of abdomen (fig. 6A) with tergum broadly produced medially; sternum narrowly cleft medially. Genital vesica and apodemes 0.39 mm. long.

Genitalia (fig. 2D, E) with a single pair of large claw-like penis valves; penis slender, elongate.

Female: Total length 2.14 mm.; wing length 2.24 mm. Color similar to male but somewhat paler and lacking yellow on abdomen. Supra-alar setae 7; subspiraculars 6; pedicelars 3. Otherwise as male is color and chaetotaxy, except for somewhat longer setae on abdomen.

Genitalia (fig. 5B) with sternum 8 deeply emarginate medially; tergum 8 large, emarginate on anterior and posterior edges and bearing a pair of small spiracles; cerci large.

Comments: The male genitalia of this species show structural affinities with the species included in the *Fuscipes* group. The structure of the rostrum, the size of the labellae and the wing venation show closer relationships to the species of the *Collaris* group.

Specimens examined: Holotype: ♂, Lake Placid, New York, July 28, 1929. A. L. Melander. Allotype: ♀, same data. Types in the collection of A. L. Melander.

Scatopse fuscipes Meigen

Scatopse fuscipes Meigen, 1830. Syst. Besch., 6: 314.
Scatopse simplex Walker, 1846. Insecta Brittanica, Diptera, 3: 144 [*fide* Edwards, 1925].
Scatopse recurva Loew, 1846. Linnaea Entomologica, 1: 330 [*fide* Kertész, 1902; Edwards, 1925; Duda, 1928].
Scatopse fenestralis Skuse, 1889. Proc. Linn. Soc. N. S. Wales, (2)3: 1384. *New synonymy.*
Reichertella peruana Enderlein, 1912. Zool. Anz., 40: 275 [*fide* Edwards, 1925; Duda, 1928].
Coboldia formicarum Melander, 1916. State Coll. Wash. Ag. Exp. Sta. Bull. 130, pp. 17–18. *New synonymy.*
Rhegmoclema barrus McAtee, 1921. Proc. Ent. Soc. Washington, 23: 123. *New synonymy.*
Reichertella capensis Enderlein, 1923. Stettiner Ent. Zeitg., 84: 67 [*fide* Duda, 1928].
Reichertella tunesica Enderlein, 1926. Zool. Anz., 68: 138–9 [*fide* Duda, 1928].
Reichertella acuticornis Enderlein, 1926. Zool. Anz., 68: 139–40 [*fide* Duda, 1928].
Reichertella algerica Enderlein, 1926. Zool. Anz., 68: 139 [*fide* Duda, 1928].
Massatierra ferruginea Enderlein, 1940, *in* Skottsberg, Nat. Hist. Juan Fernandez, 3: 666. *New synonymy.*
For other references see Kertész, 1902, and Duda, 1928.

Males: Total length 1.34–2.14 mm. Color dark grey-brown to nearly black, dull. Head jet black; antennae dull black with silvery

pubescence; setae yellow or yellow-brown, except major thoracic setae black; seventh tergum of abdomen sometimes yellow or orange brown; halteres grey-brown; legs concolorous with body, except tarsi yellowish-brown, lighter than tibiae.

Maxillary palpi 0.10–0.13 mm. long, rather slipper-shaped. Rostrum somewhat less than one-half of total head height (inc. rostrum); labellae small. Enlarged supra-alar setae 5 to 9; subspiraculars 7 to 11; pedicelars 2 to 6. Spiracular sclerite larger than in *S. notata* (fig. 3D). First tarsal segment 1.75–2.60 times as long as second. Wings (fig. 6F) clear; 1.30–2.07 mm. long; section 2 of costal margin 0.41–0.52 times as long as section 3; M 0.45–0.62 times as long as M_2; R_3 with a single, regular row of setae dorsally and a very sparse row ventrally.

Segment 7 of abdomen as in fig. 5F. Tergum produced into a long process, rather variable and usually asymmetrical. A small, lightly sclerotized tergum 8 located under tergum 7 (fig. 6G). Genital vesica and apodemes 0.20–0.25 mm. long.

Genitalia (fig. 3A, B) with a single pair of appendages of uncertain homology (possibly penis valves); penis large basally, produced into an elongate spiral.

Females: Total length 1.72–2.40 mm.; wing length 1.41–1.86 mm. Supra-alar setae 5 to 9; subspiraculars 8 to 12; pedicelars 5 or 6. R_3 with a single row of setae both dorsally and ventrally. Otherwise as male in color and chaetotaxy.

Genitalia (fig. 5D) with sternum 8 produced posteriorly into two slender, contiguous processes.

Comments: This is probably the most widely distributed of all Scatopsidae, as it has apparently been carried by commerce in bulbs and tubers throughout the world. Its larvae develop in all kinds of decaying plant and animal material, but it is most often recorded from decaying bulbs and tubers, cannery and winery waste. It has also been reported from rodent and bumblebee nests as well as the nests of seagulls and kittiwakes. The larvae have been described by Lyall (1929) and by Malloch (1916).

This species is readily recognized in the male by the medially produced seventh tergum and in the females by the single pair of contiguous processes on sternum 8.

There are some complications in the name of this species. I have no doubt that this is actually *Scatopse atrata* Say (Long's Expedition 2: 367–8, 1823). However, since *S. fuscipes* has been used for this species for the last twenty-five years, it seems best to list Say's species as unrecognizable. The large number of synonyms in this group might also cause some confusion, and a comment on these synonyms is in order. The synonymy of *S. simplex* Walker is clear since Edwards had types available and that of *S. recurva* Loew has been generally accepted since Kertész Catalog was published, and, in addition, Duda saw the male type of *recurva* in Berlin. *S. fenestralis* Skuse seems obvious from the very excellent original description. *R. peruana* Enderlein was first synonymized by Edwards (1925) and seems clear from the description. The case of *Coboldia formicarum* was settled through the generosity of A. L. Melander who lent the type to me. The reduced wings of that specimen are simply the unexpanded wings of a teneral female. I have seen some paratypes of *R. barrus* McAtee, and this is also a clear synonym. I have accepted Duda's opinion on *R. capensis*, although he did not see the types. In the cases of *R. tunesica*, *R. acuticornis*, and *R. algerica*, Duda saw types in each instance so there should be no question here. The final synonym *Massalierra ferruginea* Enderlein is inexplicable. The illustrations by Enderlein and the description can be of nothing but *S. fuscipes*.

Specimens examined: Nearly 1000 specimens of this species have been examined from almost every state in the U. S., from Alaska, from all of the provinces and territories of Canada, and from England, Spain, Holland, France, Japan, Southwest Africa, New South Wales, Hawaii, and Argentina.

Scatopse nigra Meigen

Scatopse nigra Meigen, 1804. Klassif. Ins., 1, p. 106.
Reichertella pedicella Enderlein, 1926. Zool. Anz., 68: 137–38 [*fide* Duda, 1928].
For other references see Kertész, 1902; Duda, 1928.

Males: Total length 1.69–1.93 mm. Color dark grey-brown to nearly black, somewhat shining. Head jet black; antennae dull black with silvery pubescence; setae yellow-brown, except major thoracic setae black; halteres yellow-brown; legs concolorous with body, except tarsi paler owing to silvery pubescence; membrane between tergum and sternum of segment one yellow-brown. Maxillary palpi 0.12 mm. long, rather slender; rostrum nearly half the total head height (inc. rostrum); labellae small. Supra-alar setae 19; subspiraculars 11; pedicelars 7. Anterior spiracular sclerite very small (fig. 3G); first tarsal segment of metathoracic leg 1.6 times as long as second.

Wings 1.86–1.97 mm. long, clear, with posterior veins faint. Section 2 of costal margin 0.66–0.68 times as long as section 3; M 0.29–0.38 times as long as M_2; R_3 with a single, very sparse row of setae dorsally, none ventrally.

Segment 7 of abdomen (fig. 5I) partially withdrawn into segment 6.

Genitalia (fig. 3E, F) without apparent appendages, beak-like in profile; penis with a subapical enlargement.

Females: No females have been available, but according to Duda (1928) there is little difference in size, color or chaetotaxy from the male.

Comments: The structure of the male genitalia, the wing venation, the small labellae and rather long rostrum indicate a close relationship with

S. fuscipes Mg. Duda (1928) further indicates that this species is closely allied to *S. geniculata* Zett. and *S. pulicaria* Loew. These four species might be termed the *Fuscipes* group. The probability that this should be considered the type species of *Reichertella* has already been noted.

This species is widely distributed in central Europe.

Specimens examined: 2♂♂, Berlin, Col. H. Loew; 2♂♂, Stettin, Coll. G. Enderlein.

Holoplagia Enderlein

Holoplagia Enderlein, 1912. Zool. Anz., 40: 267–268.
Holoplagia Enderlein. Speiser, 1921, Schr. physik. ges. Königsberg, 61–62: 82.

Type species: *H. transversalis*, Enderlein (*nec.* Loew) = *H. lucifuga* (Loew).

The only characters used by Enderlein to separate this genus from *Scatopse* are the presence of a complete cross vein between M_1 and R_3 and the stout form of the body. There are a number of previously described species, including the two available to me, that will fit this characterization. These are: *Holoplagia brauni* Speiser (Schr. physik. ges. Königsberg, 61–62: 82, 1921), from Brazil; *S. crassicornis* de Meijere (Tijdschr. voor Ent., 56: 326, 1913), *S. crassicula* de Meijere (Tijdschr. voor Ent., 56: 325, 1913), and *S. pallidicornis* de Meijere (Tijdschr. voor Ent., 56: 326, 1913), all from Java; *S. parallelinervis* Duda (Konowia, 7: 269, 1928), from New Guinea; *S. bullata* Edwards (Ann. Appl. Biol., 12: 273, 1925), from England; *S. transversalis* Loew (Linnaea Ent., 1: 336–7, 1846), from Europe; and *S. albitarsis* Zetterstedt (Dipt. Scand., 9: 3408–9, 1850), from Sweden.

Enderlein assigned *S. transversalis*, *S. lucifuga*, and *S. albitarsis* to this genus. Séguy (1940) added *S. bullata* and Speiser described *H. brauni* and indicated that de Meijere's species also belonged here.

On the basis of the two species that I have available (*H. lucifuga* (Loew) and *H. guamensis* (Johannsen)) this genus is quite close to *Colobostema* treated previously. It has the same short, stout thorax; the same stout, coarsely setaceous legs; and the same form in the anterior spiracular sclerite (fig. 7F, G). In addition, the antennae are longer than the head height and ten-segmented. The rostrum is short, and the labellae and maxillary palpi are small. Finally, the wings in both are densely covered with microtrichia resulting in a dull brownish or greyish appearance.

H. lucifuga and *H. guamensis* are holoptic in both sexes (whereas *Colobostema* is dichoptic in the females), have a complete cross vein between M_1 and R_3 (rather than a sharp angle on M_1 at the same point) (fig. 7I, K), and quite distinctive male and female genitalia (fig. 7A–E).

Since it seems possible that there are nine known species in this genus and only two are available to me, I do not feel justified in describing the genus further.

Holoplagia lucifuga (Loew)

Scatopse lucifuga Loew, 1870. Zeit. für die Gesamt. Naturwiss., N. folge, 1(35): 2.
Scatopse lucifuga Loew. Kertész, 1902, Catalogus Dipterorum, 1: 131.
Holoplagia transversalis, Enderlein (*nec.* Loew), 1912 (*pro parte*) Zool. Anz., 40: 268.
Holoplagia lucifuga (Loew). Enderlein, 1912, (*pro parte*) Zool. Anz., 40: 268.
Scatopse lucifuga Loew. Duda, 1928, *in* Lindner, die Fliegen der Palaearktischen Region, Bd. 2, fasc. 5, p. 28.

Males: Total length 1.80–1.90 mm. Color grey-brown, rather dull; setae all brown, except major thoracic setae black; head and thorax concolorous, grey-brown; abdomen somewhat darker; antennae slightly darker than head; legs somewhat paler than thorax. Thorax and abdomen very stout and broad. Anterior femora very stout, making the legs appear prehensile.

Maxillary palpi 0.10 mm. long, ovoid; rostrum short, labellae small. Enlarged supra-alar setae 7; subspiraculars 8; pedicelars 5. First tarsal segment 1.83 times as long as second. Anterior spiracular sclerite as in fig. 7G.

Wings 1.72–1.88 mm. long; section 2 of costal margin 0.77 times as long as section 3; M 0.37 times as long as M_2; a complete crossvein between M_1 and R_3; setae present, as usual, on costa, R_1 and R_3 but also dorsally and ventrally on the base of M basad of its fusion with R_3; R_3 with a single row of setae both dorsally and ventrally.

Segment 7 of abdomen as in fig. 7H. Genital vesica and apodemes 0.34 mm. long.

Genitalia (fig. 7A, B) asymmetrical, with one gonocoxite long and slender, the other short; penis valves large, one mandible-like, the other longer; penis large, slender and assuming an open spiral form. The asymmetry is present in the three males examined.

Females: Total length 1.85 mm.; wing length 1.55 mm. Wings slightly produced at apex (between M_1 & M_2) and covered very thickly at that point with microtrichia (fig. 7I). Otherwise as male in color and chaetotaxy.

Genitalia (fig. 7D) with a pair of small appendages on sternum 8; cerci small.

Comments: This species is present in central Europe. The types are in the Zoologische Museum der Humboldt Universität zu Berlin.

Specimens examined: 1♂, *Type*, Breslau Scholtz, Col. H. Loew; 1♂, Wien, Austria, June 25, '78, Mik; 1♂, 2♀♀, Tatra [Mts., Hungary?], Col. H. Loew.

Holoplagia guamensis (Johannsen), new combination

Scatopse guamensis Johannsen, 1946. Bull. Bishop Mus. Hawaii, 189, p. 187.

Males: Total length 1.28–1.55 mm. long.

Color very dark brownish black, dull; setae yellowish brown, except major thoracic setae black; head jet black; antennae dull black with silvery pubescence; wings with a slight brownish or greyish cast; halteres grey-brown to black; legs concolorous with body, except tarsi bright yellow to yellow-brown.

Maxillary palpi 0.07 mm. long, small, ovoid; labellae small; rostrum short. Enlarged supra-alar setae 6 to 8; subspiraculars 3 or 4; pedicelars 1. First tarsal segment of metathoracic leg 1.60–1.80 times as long as second. Spiracular sclerite as in fig. 7F.

Wings (fig. 7K) 1.04–1.24 mm. long; section 2 of costal margin 0.56–0.60 times as long as third; M 0.47 times as long as M_2; R_3 with a single row of setae dorsally; a complete crossvein between M_1 and R_3.[2]

Tergum of segment 7 (fig. 7J) with a median, longitudinal suture. Genital vesica and apodemes 0.25 mm. long.

Genitalia (fig. 7C) with gonoxites large, somewhat spatulate, penis valves small. Genitalia apparently not rotated.

Females: Total length 1.62–1.74 mm.; wing length 1.17–1.28 mm.; 2–3 setae on pedicel of halter; abdominal tergum 7 with a shallow, median cleft on posterior margin. Otherwise as male in color and chaetotaxy. Genitalia (fig. 7E) with tergum 8 reduced; cerci large and protuberant; sternum 8 much larger than in previously described species; no appendages on sternum 8.

Comments: This species has been well described and figured already by Johannsen. Despite the name this species is tropicopolitan as will be noted in the specimen record below.

It is possible that this species is identical with *S. parallellinervis* Duda from New Guinea or one of de Meijere's species from Java.

Specimens examined: 1♂, Honolulu, HAWAII, Dec. 5, 1945; 1♂, Honolulu, Oct. 1952; 1♂, April 1951; 1♀, Moen Is., TRUK, April 1, 1946; 1♂, Colon, PANAMA, Aug. 1, 1946; 1♀, Summit, Panama, C. Z., Dec. 1946; 1♂, Accra, GOLD COAST, Africa, Sept. 9, 1943.

[2]This is not shown in Johannsen's figure.

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REACTIONS TO SOUNDS BY THE WOOD NYMPH BUTTERFLY, CERCYONIS PEGALA[1]

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INTRODUCTION AND REVIEW OF LITERATURE

That some species of moths produce and receive sounds is now well established. Réaumur (1734, pp. 294–295; 1736, pp. 289–293) first called the attention of scientists to the sound produced by the Death's Head Moth, *Acherontia atropos*. Many papers on the sounds produced by this and other species of moths have since appeared

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(cf. Swinton, 1880, pp. 118–127; 205–206 for review of earlier work; Frings, 1955 for refs. to lit.). Moths are also known to receive sounds by means of an auditory organ first described by Swinton (1877, 1880, pp. 243–249). Experimental studies on sound reception by moths and the physiology of the tympanum have been fairly numerous also (cf. Eggers, 1925, 1928 for review of earlier work, Kennel and Eggers, 1933, Schaller and Timm, 1949, 1950, and Treat, 1955 for more recent studies).

Production of sounds by butterflies has not been so extensively observed. Darwin (1839, p. 38; 1845, p. 33) reported the production of sounds by *Ageronia feronia*, a tropical nymphalid, and thus seems to be the first to draw the attention of scientists to this. Swinton (1880, pp. 112–118, 205; 1889) reviewed the earlier reports and added some observations of his own. Edwards (1889), Hampson (1892), Japha (1905), Comstock (1925, p. 750), and Wilks (1940) also reported on sounds produced by various nymphaloid butterflies. The significance of these sounds has not been determined, nor the methods of production, but the general assumption has been that the sounds are used in courtship or to scare predators.

The reception of sounds by butterflies has been poorly studied. Rothke (1909) seems to have been the first to note that a butterfly, *Limenitis arthemis*, responds to sounds. Unfortunately, the sounds he used were produced by slapping and banging objects against floors or table tops, and the possibility of stimulation by gross vibrations through the substrate or by massive air movements was not ruled out. Hamann (1909) expressed the opinion that butterflies did not respond to sounds, based on observing them near railroads, and other sources of loud noises. Richter (1910) criticized both of these on the basis that Rothke did not rule out vibrations or air currents and that Hamann was discussing sounds of no natural significance to the organisms. Rothke (1912), in answer to Richter, claimed that he did not have vibrations or air movements in his tests, but he offered nothing new. Collenette (1928) noted that some individuals of *Ageronia* sp., the butterflies observed by Darwin and others as sound-producers, answered clicking sounds produced by warblers and suggested that they must therefore receive these sounds. Autrum and Schneider (1948) have recently shown that the subgenual organs of nymphalid and satyrid butterflies are sensitive to minute vibrations of the substrate on which the legs are placed, thus indicating that the reactions noted by Hamann could have been due to vibrations reaching the insects through the feet

There have been a number of morphological studies on possible sound-receptors in butterflies, but these have not been supported by experiments and only indicate possible receptors. Graber (1882) described scolopidial organs in many insects including Lepidoptera and postulated that these were for sound-reception in all orders. Guenther (1901) studied histologically sensory scales on the wings, and Vogel (1910, 1911) described campaniform and scolopidial organs on the wings that might be receptors for vibrations. Later, Vogel (1912) described a structure at the base of the fore-wings in four satyrids that, from similarity to the tympanum of Orthoptera, he thought to be auditory in function. This view did not meet with general acceptance. Kennel and Eggers (1933) and Debaisieux (1935), for instance, stated that no tympanum had been described for butterflies. LeCerf (1926) also found this organ in many species, but believed that it was not at all suited for phonoreception. Debaisieux (1935) studied scolopidial organs in the legs of butterflies, but believed that they were not adapted for reception of sounds. As noted earlier, Autrum and Schneider (1948) have shown that the subgenual organ, at least, receives vibrations.

It seems safe to conclude that some butterflies produce sounds, but the significance of these has not been determined. The evidence on reactions to sounds is unsatisfactory. Certainly Richter's criticism of the earlier work is fair, and this leaves only the chance observation of Collenette as evidence for reception of air-borne sound. That the subgenual organs of these insects are sensitive to solid-borne vibrations is undeniable. The morphological studies merely indicate possible receptors. Only with critical experimental studies can the actual receptors for sounds be determined.

For our experiments, we used the butterfly, *Cercyonis pegala* (F.), subspp. *nephele* (Kirby) and *alope* (F.), of the family Satyridae. The nomenclature of the species is unsettled, and we give here the name used by Klots (1951, pp. 72–73). Unfortunately, as the work was being done, Vogel's paper was unknown to us, and consequently the part played by the organ which he described as tympanal was not determined. We hope that we shall be able to study this point in the near future. In the meantime, a report of the experiments seems justified as showing an unmistakable reaction by a butterfly to sounds and giving some information on possible receptors.

MATERIALS AND METHODS

The butterflies were caught in the field and were thus of unknown antecedents. In all, 25 individuals (13 ♀ ♀ and 12 ♂ ♂) were studied. They were maintained in the laboratory in a cubical screen cage, 40 cm. on a side, where they were given water and sugar-water. Under these conditions they lived for 3–4 weeks, and the females deposited many eggs.

For testing, the butterflies were placed singly in smaller cubical cages, 15 cm. on a side. These

were supported on a rack made of 1 cm. mesh hardware cloth in a small anechoic chamber. The chamber was made of two boxes, one inside the other, the inner being 60 X 45 X 45 cm., the outer 90 X 80 X 80 cm. The inner box was separated from the outer by loose cotton batting, and both were made of rough, porous wall-board.

The sounds were "pure" tones from 350–20,000 Hz. produced by a Hewlett-Packard Model 200 A audio-oscillator activating a Jensen Type NF 101 loud speaker for frequencies below 4,000 Hz. and an Altec 633A microphone for frequencies above 4,000 Hz. The transducers were about 15 cm. from the testing cage, suspended by thin rubber bands from the ceiling of the chamber to reduce conduction of vibrations to the walls to a minimum. The walls were loosely joined, and this along with support of the testing cage on the very flimsy mesh rack would seem to reduce conduction of vibrations to the testing cage to nearly zero.

Control of sound pressures was obtained by using the attenuator of the oscillator. Sound pressure levels in the cage were measured with a Scott sound level meter, Type 410 B, for which a calibration curve had been made from 100–8,000 Hz. Sound pressures at 10,000 Hz. were estimated from earlier measurements. Above 10,000 Hz. sound pressures could not be measured by the available equipment. The tones produced by this system are "pure" only in the sense that most of the sound energy is in the fundamental frequency. The objectives of the experiments did not justify elaborate and time-consuming studies of the sound-fields.

The variation in sound pressure across the testing cage was about 10 db. Generally tests were made with the butterflies at approximately the same place in the sound field, the cage being moved carefully to bring the animals to that point. It is safe to assume, however, that there was a difference of about ± 3–4 db. in different tests.

For later experiments designed to localize the receptors more accurately than could be done with unrestrained animals, the insects were fixed by the ventral side of the thorax to wax blocks on the ends of glass rods, like the method described in Frings (1947). Thus mounted (Fig. 1), they could be held at any desired place in the sound field.

EXPERIMENTS AND RESULTS

When undisturbed in a cage these butterflies held the wings vertically upward and together, the anterior two-thirds to three fourths of the fore-wings visible, the remainder behind the hind wings (Fig. 1). On the under side of the fore-wing near the distal margin there are two darker spots in a lighter area. When stimulated by sound the fore-wings were drawn sharply posteriorward behind the hind wings. This reaction was clear-cut and easy to observe. If the sound was continued, the fore-wings were held behind the hind wings. If the sound was turned off, the fore-wings were gradually raised until the spots again showed. This usually occurred in less than a minute after the sound was turned off, but in some individuals the erection of the wings was much slower. It could be hastened, in these cases, by tapping or scraping on the cage near the animal.

This reaction made a good index of reception, and it was used for most of the experiments. Other reactions to sounds also took place. The

FIG. 1. Living *Cercyonis* mounted on glass rod by fastening the ventral surface of the thorax in a block of paraffin on the rod after removal of legs. 1½ X natural size.

abdomen was lifted, usually simultaneously with the dropping of the fore-wings. In most of the individuals, the antennae were flicked downward as the sound was turned on. In a few individuals, these primary reactions were followed by movement or flying. In this case, it was often 5 to 10 minutes before the insect settled sufficiently for retesting. There was considerable individual variation in the secondary reactions, and thus some individuals were much more suitable subjects for testing than others. One or two of the individuals were so prone to fly or walk for some time after stimulation that they were only tested for a few trials. On some days a few of the females were occupied with oviposition and moved

about constantly, thus interfering with testing. Otherwise, no differences in reactions between males and females were noted.

The retraction of the wings was consistently obtained at full output of the oscillator over a wide range of frequencies: from 800–10,000 Hz. Knowing that these animals probably had excellent vibration receptors in the legs (Autrum and Schneider, 1948), we tested them in two types of cages, one made of fly-screening, the other of cloth mosquito-netting on a frame made of dowels. To change the resonance conditions of the cages, they were of different sizes and the netting was drawn to different degrees of tautness. Reactions under all these circumstances were the same, and the intensity thresholds, where determined, were similar at all frequencies.

TABLE I

NUMBER OF INDIVIDUALS RESPONDING, OUT OF 18 TESTED, AT FULL OUTPUT OF SOUND SYSTEM AT SELECTED FREQUENCIES

FREQUENCY (HZ)	SOUND PRESSURE (DB)	NO. RESPONDING
100	82	0
150	85	0
200	90	0
300	98	0
350	101	0
400	105	1
500	105	3
600	109	9
800	117	14
1000	111	17
1500	116	18
2000	122	18
3000	118	18
3500	113	18
4000	108	17
5000	109	17
6000	115	16
8000	104	15
10000	106	13
15000		0
20000		0

If vibration of the cage were involved, there should be different degrees of vibration with different sizes and conditions of construction. Later experiments with mounted butterflies, in which all the legs were removed before mounting, gave essentially the same results as with free animals. Further, when the legs were actually vibrated, as in tapping or scratching on the cage, the insects responded by moving the wings forward, not backward, as they did when stimulated by sound. It seems fairly certain, therefore, that these animals were responding to air-borne sound.

The next series of experiments was made to determine the frequencies and intensities inducing this reaction in caged but otherwise unrestrained insects. With a butterfly in the cage, and the oscillator set to give maximum output, the insect was tested at the frequencies listed in Table I. At each frequency the sound was turned on suddenly, held for about 2 seconds, then turned off. The reaction, if any, was noted, and the animal given about 1 minute rest. During this time, the fore-wings, if depressed, were usually returned to the normal position. If, at the end of 1 to 2 minutes, the insect had not brought the wings up, the cage was tapped to induce the erection, after which a rest period was allowed and the next frequency was tested. Ordinarily testing was first in ascending order of frequencies, followed by descending order. Table I presents the results.

This shows clearly the wide range of frequencies evoking the response. The lower limit could obviously be determined either by frequency or intensity, because the intensity dropped off markedly below 350 Hz. The upper limit, which for most of the butterflies was between 11,000 and 14,000 Hz., might also be determined by frequency or intensity. Our equipment did not allow measurements of sound pressures above 10,000 Hz., but we know that the sound pressure output of this microphone transducer drops off above 10,000.

The third series of tests was designed to determine the intensitities needed to induce the response. Testing was as before, except that at each frequency the attenuator was set at the minimal level for the first test, and the sound level was increased by small steps, with a test of about 2 seconds duration at each setting, until the animal responded. This was repeated at least once for each butterfly for each frequency at each testing period and the value obtained was considered as the threshold for that period. The animals were given three sets of trials like this to arrive at the final values. The results are presented in Table II.

Keeping in mind that there was a variation of about 5–8 db. inherent in the testing situation, it is obvious that only at 800 Hz. is there a difference from other frequencies that might be significant, and even this is not certain. The shift in threshold from 105–109 db. between 1,000 and 3,500 Hz to 98–101 db. above 4,000 is probably related to the shift in transducer, for the loudspeaker was used for frequencies below 4,000 and the microphone for that and above. Why this should be so is not apparent, but undoubtedly this phenomenon is related to the difference in propagation patterns of the two transducers. It emphasizes the fact that these values must not be regarded as accurate determinations. We regard them as useful for indicating the general order of magnitude of sound pressures needed to evoke the reaction: 95–115 db., and for comparisons between animals under different conditions. Thus, the thresholds in the metal screening cages were almost exactly

the same for the same individual as in the cloth cages.

Busnel (1955) has suggested that reactions of insects to artificial sounds are released chiefly by transients in the stimulating sounds. In the experiments discussed so far the sudden turning on of the sound resulted in a transient system. To check this hypothesis, we tested the butterflies at 800, 1000, 2000, 3500, 5000 and 10,000 Hz. with gradually increasing sound pressures. The attenuator of the oscillator was set at the minimal value and the sound turned on and left on. The attenuator dial was turned slowly so that the intensity increased gradually. About one minute was taken to bring the attenuator from the minimal value to near the threshold previously determined for the individual. In most cases, the animal responded at nearly the same sound pressure by dropping the fore-wings. This, along with the observation that the wings remained depressed for as long as 2 minutes if the sound remained on but not when it was turned off, indicated that this reaction may not require transients. However, repeated short bursts of sound at subliminal intensities or with non-reactive animals often elicited responses.

TABLE II

MEAN SOUND PRESSURE THRESHOLDS FOR RETRACTION OF WINGS BY ADULT *Cercyonis* AT SELECTED FREQUENCIES. THE STANDARD ERRORS ARE COMPUTED BY DIVIDING THE STANDARD DEVIATIONS BY THE SQUARE ROOT OF THE NUMBER OF ANIMALS TESTED.

FREQUENCY (HZ)	NUMBER OF ANIMALS TESTED	NUMBER OF THRESHOLD DETERMINATIONS	MEAN SOUND PRESSURE THRESHOLDS (DB) ± STANDARD ERROR	RANGE OF THRESHOLDS (DB)
800	11	27	115±1.0	106–117
1000	11	32	107±1.1	97–111
1500	12	33	109±1.0	100–115
2000	13	36	108±0.9	103–113
3000	13	37	106±0.9	101–113
3500	12	38	105±1.0	97–110
4000	13	37	101±0.7	96–107
5000	13	37	99±0.7	95–105
6000	13	36	104±1.0	95–109
8000	13	36	96±1.0	91–104
10000	13	33	102±0.8	97–106

The fourth line of study was an attempt to locate the receptors involved. For this, the animals were variously mutilated and thresholds determined after recovery from the operations. On the hypothesis that the antennae were involved, a variety of extirpations were made: removal of the whole right or left antenna, removal of one-half or two-thirds of both antennae, total removal of the antennae, and covering the bases with paraffin. None of these operations affected the reaction in the least. The palpi were then removed, along with the antennae, but again without effect.

On the assumption that the sound might be received by the subgenual organs—in spite of the evidence to the contrary—the legs were totally removed and the animals mounted on paraffin blocks as described previously (Fig. 1). This too had little effect, except that two individuals thus mounted responded at 300 and 350 Hz. as well as the other frequencies to which all responded. In general, the reactions of the mounted individuals were more clear-cut and occurred at slightly lower intensities than those of free animals. We believe that this was probably due to the fact that they could be observed better when fixed.

The antennae and palpi were thereupon removed from the mounted animals, but without effect. Various operations were then performed on the wings, finally resulting in almost total removal, but, in all cases, the organ described by Vogel was present. The last, of course, necessitated using an index of reception other than that used before. The raising of the abdomen on stimulation by the sound was used. With the legs, antennae, palpi and most of the wings removed, the thresholds were a little higher, but this may have been due to the change in criterion of response. At any rate, receptors were still present under these conditions.

Returning to new individuals with the legs removed for mounting, the abdomens were ligated and removed. This too had no observable effect on the thresholds. Tests on the isolated abdomens could not be made, because these gave no visible reactions even to extreme stimulation, pricking or the like.

The heads were thereupon removed from animals that lacked the abdomens, and again the reactions were not noticeably affected. These thoracic fragments lived for 2 to 4 days and most of them continued to respond to sounds for that time. The wings of these fragments were variously mutilated, always leaving at least one-tenth of each to have a visible response, without affecting the reaction noticeably.

The isolated heads resulting from these operations were mounted on paraffin blocks and tested similarly. These responded by flicking the antennae, as noted in the intact animals at frequencies from 800–4000 Hz. A few also uncoiled the proboscides if the sound were allowed to remain on for more than about 5 seconds. This last reaction is significant, because it did not occur immediately, but developed only with continued stimulation.

Some individuals were mounted and had the heads removed. As expected, these proved to be as sensitive as normals. They lived for 3 to 5 days. After the first two days, they became rather unreactive. If the wings of one of these

were forced down at the sides until they were below the horizontal, they remained in this unnatural position. When stimulated by sound the wings were sharply drawn together in the usual erect position. After this, they could be reset and retested. There was a seeming increase in sensitivity under these conditions. This may have been due to better exposure of the receptors by thus spreading the wings, to the nature of the response, or to the unnatural position at the start. Intact animals would not hold the wings this way.

The following operations, therefore, had no apparent effect on reception of sounds: removal of legs, removal of antennae, removal of palpi, mutilation of wings or even removal almost to the bases, removal of the head or abdomen or both, or any combination of these. The thorax alone was as sensitive as the intact animals and the head nearly so. The abdomen could not be tested separately. About 1530 tests were made in the experiments on thresholds and localization of receptors.

DISCUSSION

These experiments show that butterflies of this species respond to air-borne sounds. The reaction seems to be dependent more upon the attainment of sufficient intensity—about 100–110 db.—than upon the frequency. Stimulation by vibrations arriving through the substrate seems to be ruled out by the fact that the reaction was obtained at essentially the same frequencies and intensities under a wide variety of different conditions and with the legs removed.

It is not possible to say what receptors are involved in this response. An organ like that described by Vogel (1912) and LeCerf (1926) is present on the ventral side of the bases of the fore-wings. As noted before, these papers came to hand only after this study was completed. This organ was, therefore, present in all the specimens tested, except isolated heads. That it could be the receptor cannot be denied, although Le Cerf's morphological studies led him to believe that the organ is not phonoreceptive, because the membrane is not capable of vibration. Only critical experiments can settle the question. In the meantime, we favor the hypothesis that this organ is not involved.

Among other possible receptors are tactile hairs, campaniform organs, scolopidial organs, or muscle proprioceptors (Finlayson and Lowenstein, 1955) stimulated by vibrations set up in the body-wall. The reaction is like that reported by Minnich (1925, 1936) for caterpillars, likewise released by relatively high intensity sounds. Minnich's data indicate that tactile hairs are the receptors in caterpillars, and we are inclined to believe that these widely distributed organs are active in *Cercyonis* adults. These would easily be stimulated by movements of air particles in high intensity sound fields.

Further studies are needed to determine the mechanism of reception and the possible biological significance of the reaction. The fact that reactions can be obtained from Lepidoptera without a fully developed tympanum like that of noctuid moths may explain the reactions obtained from moths in which the tympanum is destroyed (Stobbe, 1911; Eggers, 1925; Treat, 1955). In these cases, the need for relatively high intensity, as these authors report, may simply mean that reception has been shifted from the tympanum to other receptors on the body. This emphasizes the need for specification of intensities and frequencies of sounds used in experiments on sound reception if the data are to be meaningful.

SUMMARY

Adult wood nymph butterflies, *Cercyonis pegala*, reacted to "pure" tones of known frequencies generated by an audio-oscillator by moving the antennae, dropping the erected fore-wings behind the hind wings, or raising the abdomen. Using these reactions as indices of reception, intensity thresholds for frequencies of 400–20,000 Hz. were determined. The sound pressure needed to elicit the reactions was relatively high: 95–105 db. Frequency did not seem to be important, provided the intensity was sufficient. Isolated heads responded with movements of the antennae and proboscis. Isolated thoraces, without legs and minus almost all of the wings, mounted on wax blocks, responded like normals. The tactile hairs on the bodies may be the sensilla that are stimulated, but an organ at the base of the wings that has scolopidial components may be involved.

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A PRELIMINARY STUDY OF HYALURONIDASE IN SEVERAL SPECIES OF NON-VENOMOUS INSECTS[1, 2]

THOMAS M. STEVENS

In 1929 Duran-Reynals reported that the injection of virus plus mammalian testicular products into the skin of test animals resulted in a much greater spread of the virus than occurred from the injection of virus alone. He later showed that certain pathogenic bacteria, poisonous snakes, and insects, such as bees, wasps, and mosquitoes, also produced a spreading factor (Duran-Reynals, 1933 and 1936). Meyer *et al.* (1937) isolated the mucopolysaccharide, hyaluronic acid, from connective tissue and found that it was enzymatically hydrolyzed by certain bacterial extracts. They named the enzyme hyaluronidase. In 1939 Chain and Duthie tied the work of Duran-Reynals to that of Meyer by showing that hyaluronidase and the spreading factor of Duran-Reynals were one and the same. Enzymatic spreading factors which hydrolyze hyaluronic acid were subsequently found in a variety of animals including man, although the factors from various sources were found to differ somewhat in their kinetics. Consequently, a complex of hyaluronidases was recognized rather than a single enzyme.

Among insects hyaluronidases were formerly believed to function simply as spreaders of venom or saliva in the victim; however, the frequent appearance of the enzymes in non-venomous animals, other than insects, suggested the possibility of a similar distribution among the Hexapoda. If this is so, the role of hyaluronidase cannot be merely one of facilitating the spread of venoms, but is one of more basic importance to the life processes of the insect. This paper reports the results of a study of the distribution of hyaluronidase among several non-venomous species.

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MATERIALS AND METHODS

A. Rabbit injection test

1. India ink preparation—Higgins American India Ink diluted with one part ink/2 parts distilled water
2. K_2HPO_4—Fisher Certified Reagent
3. NaOH—Fisher Certified Reagent

Two methods were used in attempting to demonstrate the enzyme in non-stinging, non-biting insects. The first (Hoffman and Duran-Reynals, 1931) is a relatively crude one, the substrate being the hyaluronic acid present in the skin and underlying fascia of the rabbit. The test solution is injected with a small amount of India ink into the rabbit skin. If the enzyme

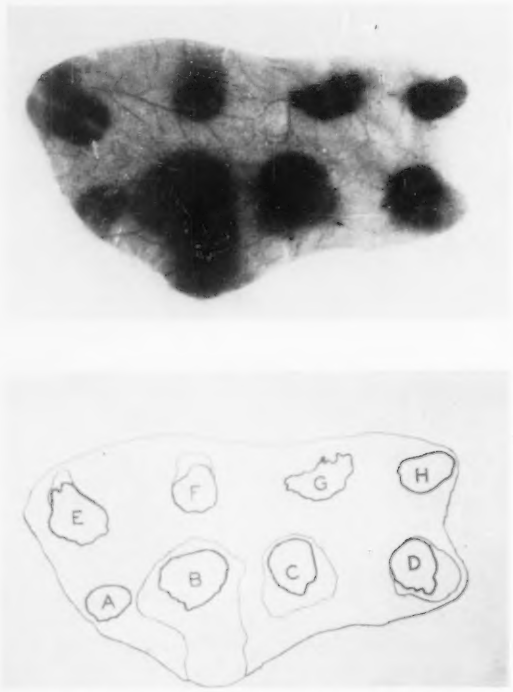

FIG. 1. Injections of a homogenate of American cockroaches in decreasing concentrations. (See text for explanation.)

is present, a depolymerization of the hyaluronic acid of the skin occurs, thus reducing the viscosity of the ground substance, and enhancing the spread of the particles of India ink through the skin.

In the present experiment the method of Hoffman and Duran-Reynals was modified as follows. Insect homogenates were prepared in a Waring blendor or Potter-Elvehjem homogenizer using 2 ml. of a 0.2 M phosphate buffer (pH 6.5) per gram of tissue. One-fourth milliliter of India ink preparation and one-half milliliter of insect homogenate were mixed in a hypodermic syringe and injected into the skin. Eight to ten injections were made in each rabbit, and the animal was killed and skinned after 16 to 18 hours. The skin was spread and dried in a forced-draft oven at low temperature.

B. Turbidity reduction test

1. Hyaluronate—Wyeth sodium hyaluronate from bovine vitreous humor
2. Hyaluronidase—Wyeth bovine testicular hyaluronidase
3. Horse serum—Difco Laboratories
4. $HC_2H_3O_2$—Baker Analyzed Reagent
5. NaOH—Fisher Certified Reagent
6. HCl—Fisher Reagent Chemical
7. $KC_2H_3O_2$—Baker Analyzed Reagent
8. $NaC_2H_3O_2.3H_2O$—Fisher Reagent, C.P.

As work progressed the need for a more refined test arose, and the turbidity reduction method (Kass and Seastone, 1944) was utilized. This test is based on the fact that purified hyaluronic acid, depolymerized by hyaluronidase, loses its ability to form a turbid, colloidal complex with the protein in acidified horse serum.

The crude insect homogenates used in the rabbit injection test were partially purified by the method of Claude and Duran-Reynals (1937). The insects, or parts thereof, were homogenized in 0.1 N acetic acid (2 ml. per gram of insect) and refrigerated overnight. The homogenate was then filtered and neutralized, and the resulting filtrate was tested for hyaluronidase activity.

The following account describes the turbidity reduction technique as it was used. Dried hyaluronate is dissolved in a 0.1 M sodium acetate buffer (pH 6), the concentration being 0.25 mg of hyaluronate per milliliter of solution. One-half milliliter of this solution, containing 0.125 mg. of substrate, is placed in a test tube, and 0.5 ml. of the tissue extract is added. The combination is incubated at 37°C. for 30 minutes, after which it is placed in ice water for 5 minutes to stop the reaction. Four milliliters of acidified horse serum are then added. In the absence of hyaluronidase a colloidal precipitate is formed which causes a measurable amount of turbidity in the solution. The degree to which this turbidity is reduced is a measure of the amount of hyaluronidase present in the tissue extract.

The acidified horse serum is prepared by diluting one volume of horse serum with nine volumes of 0.5 M potassium acetate buffer (pH 4.3). The resulting solution is reduced to pH 3.1 with 4N HCl and allowed to stand overnight. Prior to use this stock solution is further diluted with three parts of the same acetate buffer.

The test was used primarily in serial dilution studies in which each tube of the series contained a constant amount of the substrate (0.125 mg.). One milliliter of the insect filtrate was added to the first tube from which all of the others were treated in serial fashion. After the incubation

period, horse serum was added and the tubes were allowed to stand for 20 minutes before measuring the turbidity. The instrument used was a Lumetron colorimeter in which an orange filter was employed to minimize the effects of the slight yellow color of the filtrate and horse serum.

RESULTS

A. Survey of several species of insects for spreading factors

Using the rabbit injection technique, tests were made on homogenates of male and female mosquitoes, *Aedes aegypti* (L.); houseflies, *Musca domestica* L.; drone and worker bees, *Apis mellifera* L.; male and female American cockroaches, *Periplaneta americana* (L.); passalid beetles, *Passalus cornutus* F.; tent caterpillars, *Malacosoma americanum* (F.); and fall cankerworms, *Alsophila pometaria* (Harr.). With two exceptions the results were rather inconclusive. Although nearly all of the above homogenates produced a slightly greater amount of spreading than did the controls, the differences were not sufficiently larger to be considered valid indications of the spreading factor.

The exceptions were the passalid beetle and the American cockroach, both non-stinging, non-biting insects. Only one homogenate of passalid beetles was prepared due to lack of material. This homogenate and all of the roach homogenates caused extensive spreading of the India ink injections. Figure 1 shows a typical skin injection with a series of dilutions of a cockroach homogenate. A represents the control injection. B is an injection of roach homogenate, the concentration of which was 2 ml. of buffer per gram of roach. C, D, E, F, G, and H, represent homogenate concentrations of 10, 20, 60, 250, and 500 ml. of buffer per gram of roach, respectively.

Other tests on roach homogenates showed that boiling destroyed the spreading activity, whereas filtering and dialyzing did not, thus demonstrating its enzymatic nature.

B. Localization of the site of production of the cockroach enzyme

To facilitate a more detailed exploration of the spreading factor in the roach, the turbidity reduction test was employed. In an attempt to localize the site of production of the enzyme, tests were made on filtrates of the following tissues and organs: alimentary canal, salivary glands, fat body, male and female accessory glands, ovaries, muscles, nerves, testes, blood, and the carcass with alimentary canal and salivary glands removed.

Hyaluronidase activity could be demonstrated only in the alimentary canal and salivary glands. Figure 2 shows a comparison of the activities of filtrates of approximately equal concentrations (dry weight) of alimentary canals and salivary glands. All the other tissue filtrates showed no activity, although they were approximately twice the concentration of the salivary gland filtrate.

Further investigation of the alimentary canal showed that enzyme activity was concentrated, not in the walls of the canal, but in its contents. It therefore appeared probable that the enzyme was not being produced by cells of the gut but was being swallowed from the salivary glands. Another possibility was that commensal bacteria in the roach gut were producing the enzyme. However, filtrates from cultures of bacteria from the fore, mid-, and hind-guts were inactive when tested by means of the turbidity reduction method.

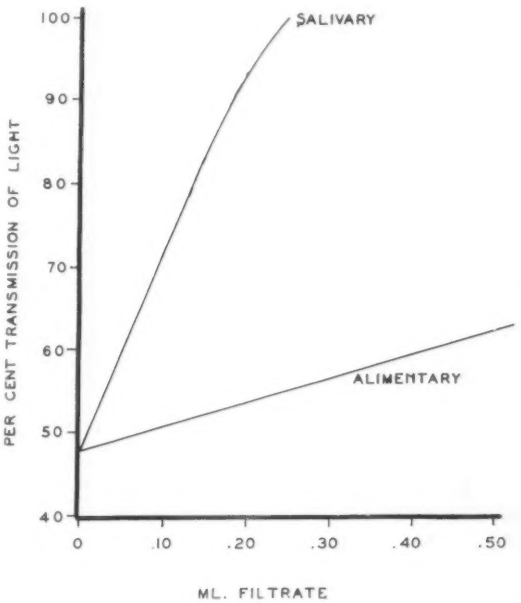

FIG. 2. Turbidity reduction of hyaluronic acid by female salivary gland and alimentary canal filtrates of approximately equal concentrations by dry weight.

DISCUSSION

The tests described above lead to the belief that the salivary glands of the American cockroach secrete hyaluronidase, which is swallowed with the food into the alimentary canal. The salivary glands seem to be the only major site of production in the roach since tests on filtrates of the other tissues and organs failed to disclose any activity.

The presence of the enzyme in fairly high concentration in the cockroach salivary glands raises the question of its function. It obviously has no defensive or offensive use, as has been postulated for stinging and biting insects and spiders. A broader role is indicated for the enzyme, unless we are to consider its presence as simply fortuitous. Perhaps it has a digestive

function or one involving permeability of the gut wall or peritrophic membrane.

Another question is whether the enzyme is universally present among insects. The rabbit injection test as performed here cannot provide an adequate answer. The injection of a whole homogenate into a living system allows any enzyme inhibitors present in the rabbit or in the insect ample time to act. Inflammatory reactions further complicate the test. Since the universality of the enzyme among insects can be regarded as a measure of its importance in their physiology, this question will receive further investigation with finer techniques.

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ELEMENTS OF ECOLOGY; by GEORGE L. CLARKE. xvi. 534 pages, illus. John Wiley and Sons, Inc., New York. 1954. $7.50.

Since the author is an eminent marine biologist it is not suprising to find that this text book has a pronounced marine flavor. That the underlying principles of ecology are little concerned with kind of life or habitat might well be the central theme of the book. In this respect it is well suited to the study of ecology. However, the insect ecologist will miss references to many of the excellent examples of the function of principles found in entomological literature. Because it is desirable to discuss and illustrate principles in terms of examples as close to the experience of students as possible, it is doubtful if this book will find much use as a text in courses where insect ecology is the prime concern. As a general reference work, it should be quite useful.

Six chapters on the physical environment provide an excellent summary of the relation of substratum, water, temperature, light and gases to organisms. A chapter on nutrients emphasizes the frequency with which growth and distribution of an organism may be seriously affected by subminimal quantities of only one of many nutrient substances. The rest of the book discusses relations among species and community aspects.

For the entomologist, the most interesting chapter is probably that concerning relations between species. The author retains the term symbiosis for the association in which one or both species are benefited and neither harmed and uses antagonism where at least one species is harmed. Following Allee, Emerson, Park, Park and Schmidt (1951) many authors now use symbiosis in the broad sense of living together and refer to the more limited concept of symbiosis as mutualism. Another term in dispute is antibiosis. As used by Allee *et al.*, this refers to a relationship of antagonism in the sense opposite mutualism. Following Clarke, antibiosis "applies more particularly to the production of materials that are specifically antagonistic to other species". Again, according to Clarke, mutualism is restricted to an association where both species derive benefit. Perhaps the student will be forgiven if he becomes a little confused in the use of these terms.

The 193 illustrations and 22 tables are well chosen to supplement and clarify the text. The index seems accurate and adequate, and of the 443 references cited, at least six were published as late as 1954.

REECE I. SAILER.

THE BIOLOGY OF THE HETEROPTERA; by N. C. E. MILLER. Pp. 162, 64 figs. Bibliography and index. Leonard Hill [Books] Lt. Stratford House 9, Eden Street, London N.W. 1. 20 s. net.

Despite the title, this book pertains mostly to the taxonomy of Heteroptera. Chapter 1 consists of a list of family and subfamily names which include the more important synonyms. Chapter 2 treats development, and subsequent chapters of Part 1 concerns legs, stridulation, natural enemies and species associated with mammals and birds. Part 2 consists mostly of descriptions for the families and the subfamilies currently recognized by most hemipterists. Excellent illustrations and photographs are provided that will facilitate recognition of most of these groups. Associated remarks treat the general habits of each group and unusual or otherwise interesting behavior of some included species.

The author takes exception to the term nymph as used for immature Hemiptera, and throughout his book refers to these stages as "neanides" a word coined by Grandi in 1951. Other entomologists have objected to the use of nymph, perhaps for the same reason as Omar Cooper, who wrote in a recent article ". . . larvae which some misguided entomologists describe as 'Nymphs', a designation which, applied to the cockroach, approaches profanity"! What ever the objection, nymph seems firmly established as a word useful in distinguishing the young of hemimetabolous insects from the larvae of holometabolous orders.

It is to be regretted that the author failed to mention the results or existence of such papers as those published by W. E. Hoffmann concerning the life history and habits of many American and Oriental Heteroptera, or that by C. O. Essenbaugh treating American Pentatomidae. Many other important works from various parts of the World could be mentioned. None-the-less, the book should prove useful, especially to the general entomologist or zoologist who needs general information concerning the appearance or habits of Heteroptera.

REECE I. SAILER.

IS SIMULIUM DINELLII JOAN (1912) (DIPTERA, SIMULIIDAE) PRESENT IN GUATEMALA?[1]

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It has been brought to the attention of the writer that, in his monograph on "The black flies (Diptera, Simuliidae) of Guatemala and their role as vectors of onchocerciasis" (Dalmat, 1955), he neglected to include under the heading "Other *Simulium* species commonly referred to in the literature as being present in Guatemala" (p. 275) the species *Simulium dinellii* Joan (1912).

It should be stated that the presence of this species in Guatemala has been indicated only by Dr. Rodolfo Robles (Calderón, 1917; Robles, 1919). After discovering the presence of onchocerciasis in Guatemala, he made an epidemiological study in the endemic region which led him to hypothesize that blood-sucking Diptera probably were the vectors. He collected two species of human-biting black flies which were sent for identification to Dr. L. O. Howard, then Chief of the Bureau of Entomology of the U. S. Department of Agriculture. Dr. Howard believed these flies to be close to *S. samboni* Jennings and *S. dinellii*, but differing somewhat from them. The specimens he examined cannot be found in the collection of the U. S. National Museum.

Although *S. samboni* does exist in Guatemala, it is not a common species, nor is it found within the altitude range in which Robles collected (Dalmat, 1955). *S. dinellii* has never been found in Guatemala. Investigators subsequent to Robles found that the three principal anthropophilic species in the onchocerciasis zone were *S. ochraceum* Walker, *S. metallicum* Bellardi, and *S. callidum* (Dyar and Shannon). Both *ochraceum* and *callidum* have some yellow to orange coloration, while *metallicum* is black. Since both *samboni* and *dinellii* also have orange to yellow coloring, it is believed that Robles' flies must have actually been *ochraceum* and *callidum*. However, there is another common black fly in the onchocerciasis zone, *Simulium downsi* Vargas, Martínez, and Díaz, 1946, which does attack man on occasion and which is very similar in external characteristics to *S. samboni*. Since this species had not yet been described at the time Robles made his collections, and since Dr. Howard did mention that one of the species he identified appeared to be *samboni* although differing from it, it is possible that what Robles called *samboni* actually was *downsi*.

Perhaps workers in Guatemala should have specifically corrected the names used by Robles, but since the identifications were not definitive, since Robles never actually worked with the flies taxonomically or from the standpoint of transmission, and since subsequent investigators did treat the taxonomy of the black flies (Bequaert, 1934; DeLeón, 1943; Vargas, 1945) without finding *dinellii* or *samboni* present in the onchocerciasis zones, these names have not been even considered.

[1]Accepted for publication Jnauary 2, 1956.

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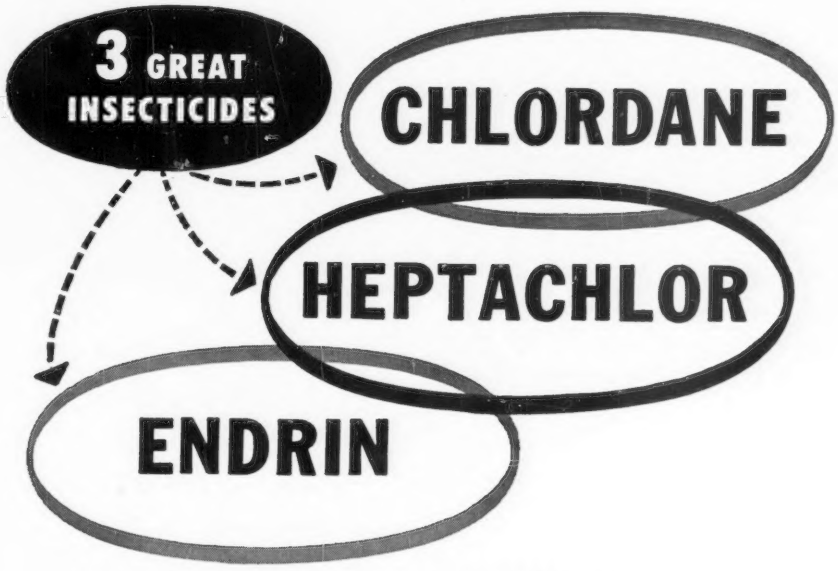

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